INDIAN AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI.

COLLECTED PAPERS

of the

Canadian Committee on Food Preservation

containing

Papers 108a, 108b
and 109 to 170

CANADA

FOREWORD TO THE FIRST VOLUME

The urgency of overcoming agricultural production problems, such as drought and disease, and the dominance of wheat among Canadian agricultural exports, have tended to relegate research on the preservation, storage, and transport of perishable foodstuffs to a position of secondary interest and attention. Of late years the volume of wheat exports has diminished and conversely the exports of meats, fish, fruits and vegetables have increased, so that for the years 1937–39 inclusive, the average annual values were, for the wheat, $107,628,000, and for the perishable foodstuffs, $85,282,000. With increasing difficulty in disposing of wheat surpluses, the trend should be to enlarge our exports of these other products to the maximum possible. Moreover, the production of most perishables being seasonal, preservation is essential to our domestic as well as to our export trade. There is thus every reason to encourage and support these institutions and scientists who have embarked upon a programme of investigations in this field.

Part of the lag in research on food preservation has been due to a lack of the experimental cold storage and other rather expensive laboratory equipment required. While this deficiency is gradually being rectified, it is likely to be a limiting factor for some time yet. It is therefore gratifying to find in the institutions concerned a disposition to pool their resources as far as this may be practicable and necessary to the realization of their common aims. This volume of Collected Papers is part of the fruits of this co-operation.

The Canadian Committee on Storage and Transport of Food grew out of a conference on cold storage held in Ottawa in June, 1934. Its general objective is to promote and co-ordinate Canadian investigations designed to increase the storage life and improve the quality of perishable products which must be stored for domestic consumption or transported to markets in other countries. To deal satisfactorily with the diversified problems coming within its scope, the Committee is organized in four sections, dealing respectively with (1) fruits and vegetables, (2) meats and meat products, (3) fish, and (4) engineering problems. Small panels for the study of taints and refrigerated railway cars have been set up under the section on engineering.

The Committee is sponsored by the National Research Council of Canada, the Dominion Department of Agriculture, and the Fisheries Research Board of Canada. The first of these organizations is studying in its own laboratories the handling and storage of meats, canning problems, and engineering problems. The Department of Agriculture, through its Experimental Farms laboratories, is investigating the processing and storage of fruits and vegetables. The investigation of problems in the preservation and transport of fish is of course a responsibility of the Fisheries Research Board. The Committee has also the co-operation of the Ontario Agricultural College, where a programme of studies on fruit and vegetable storage, designed to utilize the joint facilities of that institution, the University of Toronto, and the Horticulture Experiment Station at Vineland, is under way.

Beginning with 1937, the Committee has issued mimeographed annual reports, consisting of the summaries of the year's progress contributed by members to the annual meetings. This was intended to give to workers in the same field, particularly in other parts of the British Commonwealth, advance information on Canadian studies under way or projected, thus to encourage direct contacts and exchange of information between workers. At the same time the Committee initiated a numbered series of papers, reprints of which can be bound together in volumes to be placed in the libraries of institutions conducting food storage investigations. The number of copies of each volume to be bound is not great enough to make them available to individuals. The first volume was issued with the hope that it may prove a convenient and useful reference both to members of our own Committee and to workers in other places.

ROBERT NEWTON, CHAIRMAN,
Canadian Committee on Storage and Transport of Food.

February 5, 1941.

FOREWORD TO THE THIRD VOLUME

THE origin, sponsoring bodies and organization of the Canadian Committee on Storage and Transport of Food are described in the foreward to Volume One of these Collected Papers. The impact of war, however, diversified the activities of the Committee and stimulated investigations in other fields, particularly dehydration. In these circumstances the name of the Committee was changed to the Canadian Committee on Food Preservation and Volume Two of the Collected Papers appeared under this title.

The importance of preservation and transport of perishable foods during the war years resulted in a considerable increase in Committee activities. The sponsoring bodies assumed heavier responsibilities, and food research in university laboratories was extended through funds made available by the National Research Council for grants-in-aid of research. A new section on fats and oils was added and the Committee has now organized five subcommittees to deal with edible fats and oils, fish, fruits and vegetables, meat and animal products, and the engineering phases of food preservation and transport.

The present volume contains material that originated during the war years. A number of problems arose in connection with the feeding of Service personnel and extensive studies on ration biscuits and related items were undertaken to secure the necessary information. Wartime shortages of refrigerated shipping space, containers and packaging materials, and edible oils also brought a number of new problems to the Committee's attention. As a result most of the papers in the present volume deal with a wide variety of dehydrated foods and with packaging and substitute containers, preservation of bacon for shipment in non-refrigerated shipping space, and the conversion of linseed oil, normally an inedible product, into an edible shortening.

W. H. COOK, *Chairman,*
Canadian Committee on Food Preservation.

March 31, 1947.

TABLE OF CONTENTS

MICROBIOLOGICAL ASPECTS OF EGG POWDER[1]

C. K. JOHNS[2]

Science Service, Department of Agriculture, Ottawa

Although small quantities of spray-dried egg powder had previously been produced in Canada, the decision of the British Ministry of Food early in 1942 that all eggs should subsequently go forward in powder form created tremendous problems overnight.   The production of millions of pounds of egg powder of a quality high enough to make an acceptable dish of scrambled eggs for the British consumer some months later meant that a great deal had to be learned regarding the entire process.   That remarkable progress has been made in quality improvement is evident from the very high reputation enjoyed by Canadian egg powder in Britain; much of this can be attributed to the excellent co-operation between the Special Products Board and its technical advisers on the one hand, and the drying plant operators on the other.

Anyone who has smelt a rotten or musty egg should appreciate what bacterial growth can do to egg quality.   However, eggs used for drying are first candled and graded; then when broken out they are again carefully inspected for abnormal appearance, and smelt to detect any off-odour. With the high quality of eggs being broken for drying in Canada (84% Grade A, 16% Grade B in 1943) the initial product contains very few bacteria (6, 7, 11, 12, 15), but in a small proportion, mostly soiled eggs, bacteria have penetrated the shell.   Given favourable temperature conditions these organisms will grow rapidly and cause spoilage, hence the need for temperatures near their freezing point if eggs ear to be held for any extended period.   The broken-out eggs, commonly referred to as melange, generally contain relatively few bacteria (8); if the melange is handled in properly sanitized equipment and kept sufficiently cold before drying, the resultant powder should show a very low count. (The drying process itself may destroy as high as 99.5% of the organisms in the melange (8), although the average reduction is much less than this (2, 4).)   Unfortunately, plant sanitation and practices are not always what they should be; when conditions are not right, higher counts on the powder may be anticipated.   Consequently, as a means of checking on plant practices, limits for total viable count and for *E. coli* were incorporated in the specifications for the 1943 contract.

OFFICIAL BACTERIOLOGICAL CONTROL OF CANADIAN EGG POWDER

STANDARDS

Since little was known regarding the proper limits for bacterial content in egg powder, the standards finally agreed upon were a compromise between the views of the British Ministry of Food, the Special Products Board and its technical advisers, and the driers.   They were as follows:

Issued as Paper No. 108a of the Canadian Committee on Food Preservation.

Direct Microscopic Count Method

While the plate count method has been of distinct value in directing attention to faulty conditions, it was noticed that the powder from certain plants gave much lower counts than would be expected on the basis of sanitation surveys. It is generally agreed that the plate count of dried milk solids is not a reliable indication of the quality of the raw milk used, since the process of manufacture and subsequent storage have a direct bearing upon the extent to which the bacteria survive. Information at hand suggests that the same holds true for dried whole egg (4, 8). In the dried milk field the direct microscopic method furnishes valuable information concerning the previous history of the product (10), and it seemed logical to assume that this also holds true for dried eggs. Bartram (2) has recently reported that this is the case. Consequently, starting July 28, 1943, it was decided to make direct microscopic counts concurrently with plate counts.

The results of these comparative studies, which are being reported elsewhere (9), confirm the opinion that the direct microscopic count does afford a better indication of the care given the melange prior to drying. A direct microscopic count limit of 2,000,000 per gram is therefore being incorporated in the 1944 specifications for Canadian Grade A powder.

Coliform Organisms and E. Coli

The determination of coliform organisms or of *E. coli* in egg powder has not been found particularly helpful. In drawing up the specifications it was expected that there would be a fairly close parallel between total counts and the presence of these types of bacteria, as has been reported for frozen eggs (13). The data presented in Table 1 indicate that this is frequently not the case; a high level of coliforms may accompany a low count, or a low level of coliforms a high count. Furthermore, there seemed to be little agreement between the results of coliform tests and of plant sanitation surveys in several plants. In consequence, the determination of coliform organisms and *E. coli* in Grade A powder has been dropped for 1944.

Résumé of Results of 1943 Operations

That Canadian driers, with few exceptions, have done a fairly satisfactory job from a bacteriological standpoint is indicated by the summarized distribution of plate counts in Table 2. Only 10 (4.25%) of the 235 cars of Grade A powder exceeded the limit of 500,000 per gram. Of these 10 high counts, 3 could be attributed largely to slow freezing of melange stored for subsequent drying, with consequent bacterial development in the central core of the block; 2 others were due to the growth of a heat-resistant streptococcus in a tubular pre-heater, while only 2 could definitely be attributed to faulty care of equipment. Of the 3 remaining 2 (from Plant H) appeared to result from a peculiarity in the construction of the drier.

The high counts due to the use of a pre-heater are of interest.   This plant (G) had used the same tubular pre-heater since commencing operations in August 1942 with a satisfactorily low level of counts.   However, during February 1943 a powder cooling system was installed, whereby the powder was cooled to 80° F. or lower as it left the drying chamber.   As Gibbons and Fulton (4) have reported, prompt cooling of the powder means a significantly higher number of organisms surviving.   This, together with a gradual increase in the length of the day's run as production increased, resulted in a markedly higher level of counts.   Studies at the plant showed that counts near the start of the day's drying were quite low (14,000 to 41,000 per gram on 4 days) while at the end of the 19-hour run counts as high as 24,000,000 per gram were obtained.   This enormous increase was found to be due to the growth of bacteria in portions of the melange adhering to the rubber gaskets and to the walls of the tubes, which latter frequently showed serious scoring; the melange came from holding vats at temperatures around 35° F. and was heated to around 80° F. in the tubular heater by water at 120° F.   In at least one instance, the material remaining in the tubes at the end of the run was so acid it had coagulated.   The organism responsible for the high counts was found to be a streptococcus closely resembling *S. thermophilus.*   It produced pin-point colonies on tryptone glucose agar, and died off very rapidly in the powder.   One sample gave an initial count of 2,700,000 per gram, yet after 12 days' storage at 40° F. the count had fallen to 710,000.   Upon elimination of the pre-heater the count fell abruptly, the highest at the end of the next 3 days' runs being 7,000 per gram.

Effect of Temperatures in Transit upon Bacterial Content of Egg Powder

As was previously mentioned, the viable count of bacteria in egg powder is markedly affected by the temperature to which it is exposed after leaving the drying chamber.   In order to determine what bacteriological changes might be brought about in official carlot samples en route from the plants to the laboratory in Ottawa, two series of experiments were carried out.   In the first experiment, samples of powder were taken directly from the sifter at a local plant on 5 successive days; in the second, similarly taken samples were shipped by express from each of 6 other drying plants.   In each case the sample, after thorough mixing, was subdivided into a number of portions, each of which was exposed to a definite temperature for a definite period.   Each sub-sample was then analyzed bacteriologically and the results compared with those from the initial analysis.   From the average values, shown in Table 3, it appears that at 40° F. the bacteria counts showed no appreciable change even after 5 days; at 70° a moderate decline was noted after 2 days, being much more evident in the higher count samples, while at 86° to 90° a sharp reduction was noted, this being evident even after 1 day.   Coliform organisms and *E. coli* appeared to die off more rapidly than the general flora at all three temperatures, the decrease being particularly pronounced at the higher temperatures.

In order to keep such changes at a minimum, consideration was first given to the use of dry ice in the shipping container.   However, this had to be dropped as dry ice was not available at several points where eggs were

being dried.   Instead, it was decided to have the samples cooled as thoroughly as possible before shipment.   Samples from Western Canada were to be sent by air express, those from Eastern Canada by railway express. In this way all samples should reach the laboratory within 24 hours, with little or no change in their bacterial content.

Bacterial Flora of Melange and Powder

Some studies were conducted upon the flora of melange and of powder. Approximately 100 colonies from each of a series of plates prepared from samples of powder from 7 plants, and of melange from 1 plant, were fished into litmus milk and incubated at around 86° F. (30° C.) for 5 days.   Smears were made from each tube, stained according to Gram's method and examined microscopically.   While the flora of the single sample of melange comprised a variety of types, Gram negative rods predominated; in the powder samples on the other hand, a streptococcus which produced slight acid but failed to coagulate litmus milk was the preponderant type, often comprising over 90% of the colonies fished.
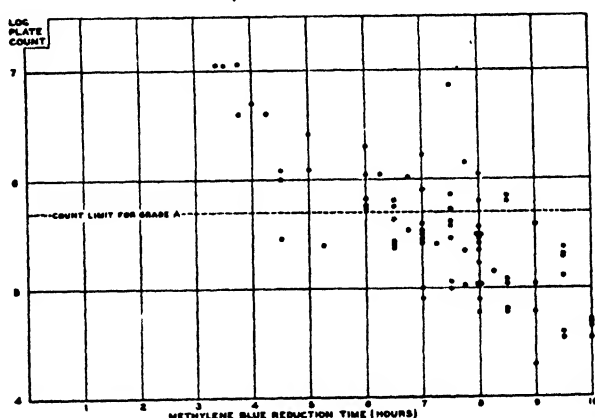
FIGURE 1.   Comparative methylene blue reduction times and plate counts on 80 samples of dried egg.

SUMMARY AND CONCLUSIONS

The standards and procedure employed in the bacteriological control of Canadian dried eggs for export to Britain are described, together with some of the results for 1943.   In 1943, out of a total of 235 carlots of Grade A powder, 95.75% gave plate counts of under 500,000 per gram.

As a simple plant test for bacterial content of melange, the Burri slant method has been found most useful.   Dye reduction tests were not so satisfactory because of end-point difficulties and the long incubation periods required.

The direct microscopic count was found to reveal the past history of the product more satisfactorily than did the plate count, coliform or E. coli test.

The possible changes in bacterial content resulting from exposure of samples to various temperatures en route to the official control laboratory were investigated and arrangements made to minimize such changes. .

Studies on the flora of melange and powder revealed that the Gram negative rods which predominated in the former were generally replaced by a weak acid producing streptococcus in the powder.

ACKNOWLEDGMENTS

REFERENCES

1. AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods for the Analysis of Dairy Products. 8th ed. New York, N.Y., 1941.

2. BARTRAM, M. T. Bacteriological changes during the dehydration of eggs. J. Bact. 45 : 48. 1943. (Abstract.)

3. DE BORD, G. G. Effect of dehydration upon the bacterial flora of eggs. J. Agr. Res. 31 : 155-164. 1925.

4. GIBBONS, N. E. and C. O FULTON Dried whole egg powder. VII. Effect of temperature and moisture on the bacterial content of liquid and dried egg. Can. J. Res. D, 21 : 332-339. 1943.

5. GORESLINE, H. E., L. S. STUART, H. F. SMART, and V. T. DAWSON. Effect of storage temperatures on bacterial counts in spray-dried whole egg powder. J. Bact. 45 : 48-49. 1943. (Abstract.)

6. HARRIS, R. B. Observations on the bacterial flora of the hen's egg, with a description of new species of *Proteus* and *Pseudomonas* causing rots in eggs. J. Hygiene, 38 : 338-355 1938

7 JOHNS, C. K. The bacterial content of shell eggs. Food in Canada. 3(12), 15-16. 1943.

8. JOHNS, C. K. Unpublished data. 1943.

9. JOHNS, C. K. and H. L. BERARD. The direct microscopic method in the bacteriological control of dried whole egg. Food Research. (In press.).

10. PRICKETT, P. S. Sanitary control of dried milk. J. Milk Tech. 2(3), 133-137. 1939.

11. RETTGER, L F The bacteriology of the hen's egg with special reference to its freedom from microbial invasion. Storrs Agr. Exp. Sta. Bul. 75. 1913.

12. ROSSER, F. T. Preservation of eggs. II. Surface contamination of egg-shell in relation to spoilage. Can. J. Res. D, 20 : 291-296. 1942.

13. SCHNEITER, R., M. T. BARTRAM, and H. A. LEPPER. Bacteriological and physical changes occurring in frozen eggs. J. A.O.A.C. 26 : 172-182. 1943.

14. SCOTT, W. J. and J. M. GILLESPIE. Tests for quality in egg pulp 1 A preliminary note on the application of the reductase test using resazurin as the indicator. J. Coun. Sci. Ind. Res. 16 : 15-17. 1943.

15. STUART, L. S. and E. H. McNALLY. Bacteriological studies on the shell egg. U.S. Egg and Poultry Mag. 49 (1), 28-31, 45-47. 1943.

16. THISTLE, M. W., J. A. PEARCE, and N. E. GIBBONS. Dried whole egg powder. I. Methods of assessing quality Can J. Research, D, 21 : 1-7. 1943

TABLE 1.—RELATION BETWEEN BACTERIA COUNTS, COLIFORM AND *E. coli* CONTENT OF CANADIAN GRADE A DRIED EGGS

Sample	Bacteria count (thousands per gram)		No. of tubes* Positive for	
	Viable	Direct microscopic	Coliform organisms	*E. coli*
A	21		3/5	3/5
B	12	220	20/20	19/20
C	41	880	15/15	11/15
D	3		12/15	10/15
E	5		11/25	8/25
F	21	880	10/20	9/20
G	55	550	15/15	10/15
H	26		2/5	2/5
I	15		5/5	2/5
J	38		8/10	7/10
K	470		0/5	
L	1,300		1/5	1/5
M	630		5/25	3/25
N	2,000		6/25	6/25
N	580		0/5	
O	1,100	12,000	0/10	
P	2,000	35,000	1/5	1/5
Q	2,000		6/25	6/25
R	1,200		2/20	2/20
S	1,100	4,100	0/5	

Inoculated with 1/100 gm. of powder.

TABLE 2.—DISTRIBUTION OF VIABLE COUNTS ON DRIED EGGS. 1943.

Grade A Plant	No. of carlot samples	Viable counts (thousands per gram)					
		<10	10–25	26–100	101–500	501–1,000	>1,000
A	20	1	14	4	1		
B	21	1	1	9	8	0	2
C	29	2	1	10	14	1	1
D	55	19	13	21	2		
E	11		2	1	8		
F	15	1	8	5	1		
G	49	15	4	16	11	1	2
H	22				20	2	
I	13	1	1	4	6		1
Totals	235	40	44	70	71	4	6
Percentage		17.0	18.7	29.8	30 2	1.7	2.6
Grade B							
A	6		1	3	2		
B	14	1	1	5	5	1	1
C	19	0	1	9	7	0	2
D	12	4	4	3	1		
E	5			3	2		
F							
G	6		1	4			1
H	5				1	0	4
I	3	1	0	2			
Totals	70	6	8	29	18	1	8
Percentage		8 6	11 4	41 4	25 7	1.5	11.4

TABLE 3.—EFFECT OF TEMPERATURE ON BACTERIAL CONTENT OF WHOLE EGG POWDER

Series I (Averages of 5 samples)	Days held	Temperature (°F.)		
		40°	70°	86°
Plate count	0	76,400	76,400	76 400
	2	84,300	55,900	30,900
	5	79,000	50,000	7,680
Coliform organisms*	0	40 4	40 4	40 4
	2	60 2	48 8	5 2
	5	53 4	20 0	0 0
E. coli*	0	20 6	20 6	20 6
	2	56 0	13 1	0 8
	5	18 4	1 7	0 0
Series II (Averages of 6 samples)	Days held	40°	70°	90°
Plate count	0	98,100	98,100	98,100
	1	100,000	90 900	52 600
	2	117,000	70 000	29,000
Coliform organisms*	0	8 4	8 4	8 4
	1	5 1	2 2	0 6
	2	4 2	1 3	0 3
E. coli*	0	1 8	1 8	1 8
	1	2 9	1 8	0 3
	2	1 8	0 7	0 0

* Most probable number calculated from inoculation of 5 tubes with 0 1 gm and 5 tubes with 0 01 gm of powder

APPENDIX A.

OFFICIAL METHODS FOR BACTERIOLOGICAL ANALYSIS OF CANADIAN WHOLE EGG POWDERS

(1) *Preparation of Sample*

Studies have shown that certain samples vary widely in count from portion to portion. Thorough mixing of the sample is therefore essential. Running the powder at least twice through a suitable type of flour sifter (which has previously been sterilized) has been found to give good results. Where the same sample is to be used for chemical analysis, care should be taken that it is not unduly exposed to the air because of the ease with which it picks up moisture from the atmosphere.

(2) *Reconstitution of Powder*

A 1 : 10 dilution is prepared by weighing out 11 grams of the thoroughly mixed sample into a sterile wide-mouthed jar or flask of approximately 200 ml. capacity containing a spoonful of glass beads. The contents of a dilution bottle containing 99 ml. of sterile physiological saline solution (0.85% NaCl in distilled water) are then poured into the jar or flask and the contents shaken vigorously until a uniform suspension is obtained, usually within a minute. The dilution should be allowed to stand for several minutes until the air bubbles have risen to the surface before preparing further dilutions.

If desired, the 1 : 10 dilution may be prepared by using 10 grams of powder and 90 ml. of saline.

Further dilutions are prepared by introducing 1, 10, or 11 ml. portions of the 1 : 10 dilution into dilution blanks containing appropriate amounts of sterile saline solution.

(3) *Escherichia coli Test*

From the 1 : 100 dilution five 1-ml. portions are pipetted into a similar number of fermentation tubes containing 2% Brilliant Green bile broth for the *E. coli* presumptive test. A 10-ml. or 11-ml. pipette graduated in 1 ml. is convenient for this purpose. Tubes are incubated at 37° C., and observed at intervals over 48 hours. As soon as gas formation is observed, the liquid in each of such tubes is streaked over the surface of a plate previously poured with eosin-methylene blue agar and allowed to harden. Two such streakings may be made on separate halves of the same plate. The streaked plate is incubated at 37° C. for 18 to 24 hours; the appearance of typical black metallic colonies is regarded as indicative of the presence of *E. coli.*

(4) *Total Viable Bacterial Count*

For total bacterial count, duplicate plates are prepared from both 1 : 1,000 and 1 : 10,000 dilutions. From the 1 : 100 dilution, 0.1-ml. portions may be transferred with aid of a 1.1 or 2.2-ml. pipette to the plates for the 1 : 1,000 dilution, while 1-ml. portions of the 1 : 100 dilution are transferred to a 99-ml. saline dilution bottle to give a dilution of 1 : 10,000. From the latter, 1-ml. portions are pipetted into plates for that dilution. If desired, dilutions may be prepared from the 1 : 100 dilution by introducing 11-ml. quantities into 99 ml. dilution blanks, or 10-ml. into 90 ml. blanks.

Approximately 10 ml. of medium, melted and cooled to 41–42° C., are poured into each plate and mixed with the inoculum within 20 minutes of preparing the 1 : 100 dilution. The medium employed is the standard medium for milk analysis, tryptone glucose extract agar containing 1% skim milk. If desired, the skim milk may be omitted, since it has no appreciable effect upon the count obtained with egg powders. After the medium has hardened, plates are incubated at 37° C. for 48 hours and all colonies counted with the aid of a Quebec colony counter or equivalent device. The average of duplicate plates on the dilution represents the total bacterial count.

Retest of Samples Exceeding Limits for Grade A

Should the total count exceed 500,000 per gram, or should 2 or more of the 5 fermentation tubes contain *E. coli*, a further 11 (or 10) gram sample is taken and the above analysis repeated. An additional 10 tubes containing Brilliant Green bile broth are inoculated with 1 ml. portions of a 1 : 1,000 dilution to ascertain whether the powder exceeds the limit for Grade B (not more than 4 of the 10 tubes to contain *E. coli*). The grade of the powder is then determined on the average of the results from both sets of analyses.

DIRECT MICROSCOPIC METHOD IN BACTERIOLOGICAL CONTROL OF DRIED WHOLE EGGS [1]

C. K. JOHNS AND H. L. BERARD

Division of Bacteriology and Dairy Research, Science Service,
Department of Agriculture, Ottawa, Canada

The sudden demand of the British Ministry of Food for enormous quantities of dried whole eggs of a quality suitable for fresh-egg dishes has introduced, among others, the problem of bacteriological control of breaking and drying operations. Since the nine drying plants in Canada are widely scattered, main reliance had to be placed upon bacteriological examination of the powder itself at a central laboratory, supplemented where possible by plant-sanitation surveys.

As a result of discussions between the driers, the British Ministry of Food, and the Special Products Board and its technical advisers the following standards were agreed upon for the 1943 drying season:

> *Grade A powder* (for use in fresh-egg dishes), total viable count on tryptone glucose agar, after 48 hours at 37°C., shall not exceed 500,000 per gram, with *E. coli* absent from 1/100 gram.
>
> *Grade B powder* (residual portions, for baking purposes only), no viable count limit, but *E. coli* absent from 1/1,000 gram.

While the plate count in particular was of value in directing attention to several cases of faulty plant practice, it was nevertheless noticed that an occasional plant, where sanitary conditions and practices were not above criticism, maintained a surprisingly low level of viable counts. Information at hand [Johns (1943a)] suggested that, as in the case of dried milk, the plate count of egg powder is greatly influenced by the method of drying and of subsequent storage. In the dried-milk field, the direct microscopic method furnishes valuable information concerning the previous history of the product [Prickett (1939)] and it seemed logical to assume that this might also hold true for dried eggs. Bartram (1943a) has recently reported that this is the case. Consequently it was decided to make direct microscopic counts concurrently with plate counts on all samples received after July 26, 1943.

The technique originally employed was to spread 0.01 ml. of a 1/10 dilution of egg powder over an area of one sq. cm. on a clean microscopic slide, then to defat, fix, and stain with the Newman-Lampert combined stain commonly used in milk work. This, however, was not too satisfactory, the background being too dark for easy counting. The acid-methylene blue stain described by Mallman and Churchill (1942) was next tried, but trouble was experienced with films washing off. Finally, the technique described in Standard Methods for the Examination of Dairy Products (1941), using North's aniline oil-methylene blue stain, was adopted with the staining period extended to one to two minutes, as suggested by Bart-

ram (1943b). This gave much more satisfactory results. We have recently been experimenting with various modifications of Gray's (1943) methylene blue-basic fuchsin stain; this also gives nice results but the technique requires more careful attention than North's. For official control purposes, at least 60 fields should be counted unless organisms are present in very large numbers. As suggested by Bartram (1943b), paired cocci are counted as a single cell to avoid attempting to differentiate between them and long rods with an unstained or lightly stained central spore; where a clump or chain of cells is found, however, each individual cell is counted.

The greater value of the direct microscopic method in indicating the previous history of the product is suggested by the data (Table 1). Plant

TABLE 1

Counts on Whole Egg Powder

Sample		Bacteria count (thousands per gram)		Ratio— D M.: Plate
		Direct microscopic	Plate	
Plant A	1	330	11	30.0
	2	220	5	44.0
	3	110	12	9.2
	4	110	17	6.5
	5	165	34	4.8
	6	330	23	14.3
	7	110	4	27.5
	8	330	30	11.0
	9	220	10	22.0
Plant B	1	550	8	69.0
	2	330	11	30.0
	3	880	21	42.0
	4	1,100	34	32.0
	5	770	9	86.0
	6	770	9	86.0
	7	440	17	26.0
	8	330	63	5.0
	9	6,200	76	82.0
	10	110	80	1.4
	11	880	91	9.8
	12	660	91	7.3
	13	950	10	95.0
	14	1,300	8	162.0

A, where good sanitary conditions prevailed, showed low counts with both methods. Plant B, where sanitation sometimes suffered as a result of emphasis upon volume of production, showed equally low plate counts (a box-type drier was used) but the direct microscopic counts were almost invariably at a much higher level than in Plant A. That this is not infrequently the case is shown (Fig. 1), wherein the relative counts by direct microscopic and plating methods on a series of 150 prime powders from nine plants are shown.

High counts in the powder may be due either to the use of low-grade eggs or to opportunities for growth of bacteria prior to drying. Since Canadian driers are furnished with high-grade shell eggs containing few

bacteria, Johns (1943b), high counts in Canadian egg powder can almost invariably be attributed to faulty plant practice. The count obtained by the plating method suffers from the disadvantage that it is significantly affected by such factors as the type of drier, drying temperature, speed of removal from drier, rate of cooling, and temperature and length of storage, so that extremely low counts may sometimes be obtained from melange with a moderately high bacterial content. The direct microscopic count, being much less influenced by the factors mentioned, reflects more accurately the conditions under which the melange was handled, and thus appears to be of greater value in control work.
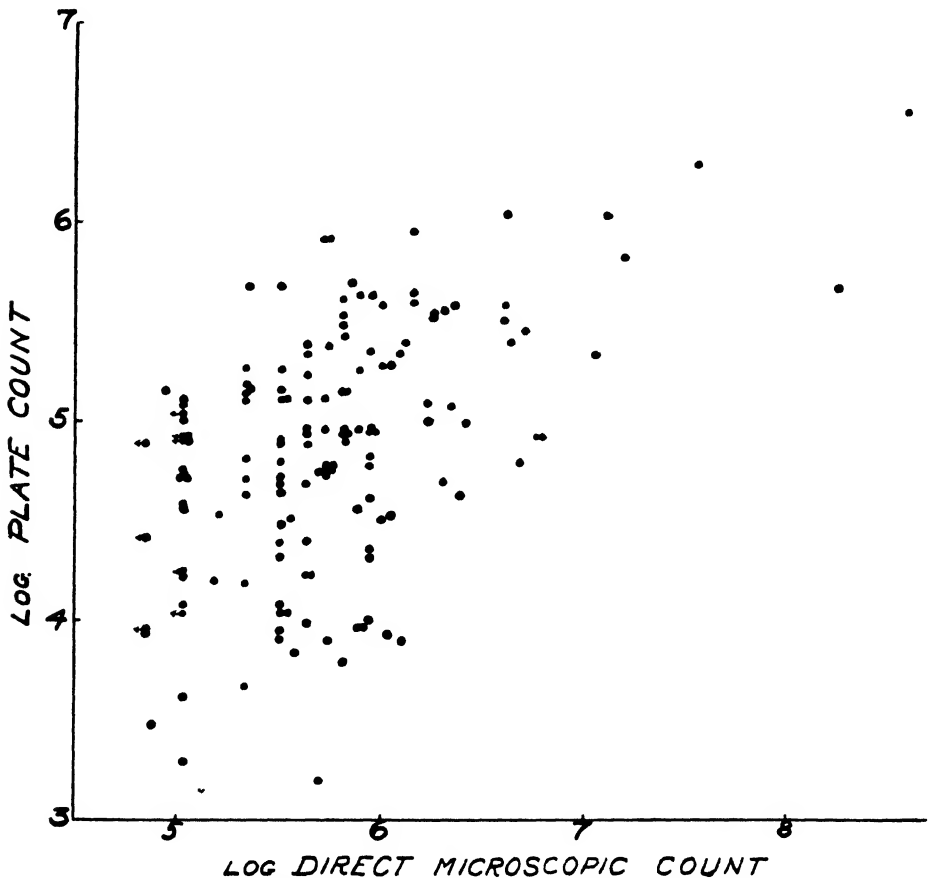
FIG. 1. Comparative counts (expressed as logarithms) of 150 samples of Canadian dried whole eggs by direct microscopic and plate count methods.

In one respect the direct microscopic method as applied to dried eggs is less satisfactory than when applied to dried milk. This is when the melange has been pasteurized before drying. While the degree of heat treatment given egg melange [60°C.(140°F.) for 30 minutes in the present instance] is less severe than that given milk in forewarming, the bacterial cells in pasteurized melange (or powder made therefrom) fail to stain

with either North's or Gray's stains as they do in dried milk. This is illustrated by the counts on samples of pasteurized and unpasteurized melange and powder (Table 2). The plant in question was working with frozen melange of such a high bacterial content that they were experiencing difficulty in meeting the bacteria count limits for Grade A powder. To reduce the count on their powder, vat pasteurization was resorted to with the result that both plate and direct microscopic counts fell to a low level. Similar results have been obtained with powder from a second plant which has followed the same practice of pasteurizing high-count melange. The possibility of so modifying the technique of the direct microscopic method that the organisms destroyed by pasteurization may be rendered stainable is being investigated.

TABLE 2

Comparison of Plate and Direct Microscopic Counts [1] on Pasteurized vs. Unpasteurized Egg

Sample	Melange in thawing tank		Melange in balance tank		Powder	
	D. M	Plate	D. M.	Plate	D M.	Plate
Unpasteurized						
Nov. 19	36,000	5,500	330,000	13,000	250,000	240
Nov. 23	46,000	8,900	240,000	15,000	52,000	630
Pasteurized						
Nov. 26	17,000	11,000	110	59	350	12
Dec. 1	3,500	900	220	8	<110	11
Dec. 16	....	1,600	<110	48	<110	33
Jan. 11	2,900	3,600	75	24	150	16

[1] Given in thousands per gram.

REFERENCES

American Public Health Association, 1941. Standard Methods for the Examination of Dairy Products. Eighth ed. New York, N. Y.

BARTRAM, M. T., 1943a. Bacteriological changes during the dehydration of eggs. J. Bact. 45, 48 (Abstract only).

———, 1943b. Private communication, Aug. 3, 1943.

GRAY, P. H. H., 1943. Two stain method for direct bacteria count. J. Milk Tech. 6, 76.

JOHNS, C. K., 1943a. Unpublished data.

———, 1943b. The bacterial content of shell eggs. Food in Canada 3, 15 16.

MALLMANN, W. L., AND CHURCHILL, E., 1942. A rapid test for determining bacteria in liquid eggs. U. S. Egg Poultry Mag. 48, 406-407, 427-429.

PRICKETT, P. S., 1939. Sanitary control of dried milk. J. Milk Tech. 2, 133-137.

Chemical Inhibition of Growth of Fish Spoilage Bacteria

BY H. L. A. TARR

Pacific Fisheries Experimental Station

ABSTRACT

Inhibition of the growth of fish spoilage bacteria in naturally contaminated fish muscle, and in some instances of pure cultures of such organisms cultivated on laboratory media, by penicillic acid, 4-methoxy-2 : 5 toluquinone, methyl formate, ethyl formate, ethylene oxide, propylene oxide, methyl ether, ethyl ether, chloroform, ethylene dichloride, ethyl chloride, 1 : 4 dioxane, Chloramine B, Chloramine T, a mixture of isomeric glycerol formals, sodium chlorite, sodium benzoate, sodium nitrite, and one patent fish preservative was investigated. The results are discussed and are summarized in detail.

The value of a number of different chemical compounds in inhibiting growth of fish spoilage bacteria in culture media and in fish flesh itself was described in previous publications (Tarr and Sunderland 1939, 1940; Tarr 1941, 1942). The bactericidal or bacteriostatic action of various chemical compounds, many of which were not previously investigated, is recorded herewith.

EXPERIMENTS WITH ISOLATED BACTERIAL CULTURES

The cultures used were those employed in previous experiments (Tarr 1939).

Penicillic acid (5-methylene 4-keto 3-methoxy 2-hexenoic acid) was prepared by the action of *Penicillium cyclopium* Westling on glucose as described by Birkinshaw, Oxford and Raistrick (1936). The *4 - methoxy - 2:5 toluquinone* was prepared by Ashley's method (1937). Solutions of these compounds of the required strength were made by dissolving them in boiling sterile distilled water (Oxford, Raistrick and Smith 1942). Sterile 16 × 150-mm. tubes received 2 ml. of Bacto nutrient broth (double the normal strength), 0.5 ml. of 0.2 M phosphate buffer (different pH values), 1 ml. of an aqueous solution of penicillic acid or 4-methoxy-2 : 5 toluquinone of the desired strength (water in controls) and 0.5 ml. of a saline suspension of cells of the organism being studied. The saline suspensions of the test organisms were prepared by transferring a portion of a 1- or 2-day-old culture of the organism in Bacto nutrient broth into sterile 0.1 per cent sodium chloride, the number of bacteria present being determined directly using a Petroff-Hausser counting chamber. The inoculated tubes were incubated at 25°C., the amount of visible growth being recorded at stated intervals.

The results of experiments using one species of *Micrococcus* and two of *Achromobacter* (tables I and II) showed that the bactericidal or bacteriostatic

257

action of both compounds varied with the cultures used, and in all instances was more pronounced in acid than in neutral or faintly alkaline medium. With the exception of culture 1 (*Micrococcus* sp.) neither compound exerted a very pronounced inhibition in 0.001 per cent concentration, 0.01 per cent being, however, usually very active in this respect. On the whole 4-methoxy-2 : 5 toluquinone exhibited similar, though more marked, inhibitory action than did penicillic acid.

Methyl bromide has marked insecticidal properties (Dudley and Neal 1942), and inhibits the growth of yeasts which contaminate dates (Mrak 1941), but as

TABLE I. Effect of penicillic acid on the growth of culture 22 (*Achromobacter* sp.), culture 16 (*Achromobacter* sp.), and culture 1 (*Micrococcus* sp.). Inocula 8.9 × 10⁶, 0.7 × 10⁶, and 2.3 × 10⁶ organisms (or clumps of cells for culture 1) respectively.

Culture no.	Initial pH of medium	Controls			0.01% penicillic acid			0.001% penicillic acid		
		1 day	2 days	2 weeks	1 day	2 days	2 weeks	1 day	2 days	2 weeks
22	5.90	+++	+++	+++	-	-	-	++	+++	+++
"	6.00	+++	+++	+++	-	-	-	++	+++	+++
"	6.20	+++	+++	+++	-	-	+++	++	+++	+++
"	6.45	+++	+++	+++	-	-	+++	++	+++	+++
"	6.75	+++	+++	+++	-	++	+++	+++	+++	+++
"	7.20	+++	+++	+++	++	+++	+++	+++	+++	+++
"	7.55	+++	+++	+++	++	+++	+++	+++	+++	+++²
"	7.80	+++	+++	+++	++	+++	+++	+++	+++	+++
16	5.90	+	+++	+++	-	-	-	-	++	+++
"	6.00	+	+++	+++	-	-	+++	+	++	+++
"	6.20	+	+++	+++	-	-	+++	+	+++	+++
"	6.45	+	+++	+++	-	+	+++	++	+++	+++
"	6.75	++	+++	+++	-	+	+++	++	+++	+++
"	7.20	+++	+++	+++	+	++	+++	++	+++	+++
"	7.55	+++	+++	+++	+	+++	+++	++	+++	+++
"	7.80	++	+++	+++	+	+++	+++	++	+++	+++
1	5.90	-	-	-	-	-	-	-	-	-
"	6.00	-	-	-	-	-	-	-	-	-
"	6.20	+	++	+++	-	-	-	-	-	-
"	6.45	++	++	+++	-	-	-	-	-	-
"	6.75	++	++	+++	-	-	-	-	-	++
"	7.20	+	+	+	-	-	-	-	-	+
"	7.55	-	-	+	-	-	-	-	-	+
"	7.80	-	-	-	-	-	-	-	+	+

far as the writer is aware its bactericidal action has not been previously investigated.

Bacto nutrient agar (pH 7.1) and nutrient broth (pH 7.2) were sterilized in 3-ml. amounts in 12 × 150-mm. tubes, the agar medium being sloped prior to inoculation. Cultures 1 to 24 were grown for 24 hours at 25°C. in Bacto nutrient broth (pH 7.0) and one 2-mm. loopful of each culture was transferred to each of five broth and five agar tubes. Five complete similar sets of inoculated media were thus obtained for each of the 24 cultures, and one set at a time was treated with methyl bromide as follows. The tubes were placed in a vacuum desiccator

and subjected to reduced pressure (about 6 cm. of mercury) by means of a water pump. The desired amount of methyl bromide (measured in cm. of mercury) was then permitted to enter the desiccator, followed by air until prevailing atmospheric pressure resulted. The tubes were exposed for 30 minutes, and were then removed, incubated at 25°C., and the presence or absence of growth noted at stated intervals. The results (table III) showed that methyl bromide exerted a pronounced bactericidal action, only 12 per cent of broth and 6 per cent of agar cultures exhibiting growth after two weeks following exposure to 50 cm. of methyl

TABLE II. Effect of 4-methoxy-2 : 5 toluquinone on the growth of culture 22 (*Achromobacter* sp.), culture 16 (*Achromobacter* sp.), and culture 1 (*Micrococcus* sp.). Inocula 8.9 × 10⁶, 0.7 × 10⁶ and 2.3 × 10⁶ organisms (or clumps of cells for culture 1) respectively.

Culture no.	Initial pH of medium	Controls			0.01% 4-methoxy-2 : 5 toluquinone			0.001% 4-methoxy-2 : 5 toluquinone		
		1 day	2 days	2 weeks	1 day	2 days	2 weeks	1 day	2 days	2 weeks
22	5.90	+++	+++	+++	−	−	−	++	+++	+++
"	6.00	+++	+++	+++	−	−	−	++	+++	+++
"	6.20	+++	+++	+++	−	−	−	++	+++	+++
"	6 45	+++	+++	+++	−	−	−	++	+++	+++
"	6.75	+++	+++	+++	−	−	−	++	+++	+++
"	7.20	+++	+++	+++	−	−	−	++	+++	+++
"	7.55	+++	+++	+++	−	−	−	++	+++	+++
"	7 80	+++	+++	+++	−	−	−	++	+++	+++
16	5.90	+	+++	+++	−	−	−	−	−	−
"	6.00	+	+++	+++	−	−	−	−	−	−
"	6.20	+	+++	+++	−	−	−	+	+++	+++
"	6.45	+	+++	+++	−	−	−	+	+++	+++
"	6.75	++	+++	+++	−	−	−	++	+++	+++
"	7.20	+++	+++	+++	−	−	−	++	+++	+++
"	7.55	+++	+++	+++	−	−	−	++	+++	+++
"	7.80	++	+++	+++	−	−	−	++	+++	+++
1	5.90	−	−	−	−	−	−	−	−	−
"	6 00	+	+	+	−	−	−	−	−	−
"	6.20	++	+	++	−	−	−	−	−	−
"	6.45	++	++	+++	−	−	−	−	−	−
"	6.75	+	++	+++	−	−	−	−	−	−
"	7.20	+	++	+++	−	−	−	−	−	+
"	7.55	+	+	++	−	−	−	−	−	+
"	7.80	−	−	+	−	−	−	−	−	+

bromide for 30 minutes. It was not determined whether methyl bromide would exert greater activity if applied in partial vacuum without allowing air to run in until atmospheric pressure was attained.

EXPERIMENTS WITH NATURALLY CONTAMINATED FISH MUSCLE

The following technique was used throughout for the sake of uniformity. Fresh fish were filleted carefully under clean conditions, the flesh was minced in a domestic machine, mixed thoroughly, and 95-g. portions were placed in sterile

250-ml. covered glass beakers. The various compounds studied (with the exception of those applied as gas) were dissolved to the desired concentration in sterile distilled water, 5 ml. of the resulting solution being added to, and intimately mixed with, one 95-g. portion of minced fish muscle. Special precautions had to be observed with solutions of 4-methoxy-2 : 5 toluquinone and of ethylene oxide. The former, which is sparingly soluble in cold water, was added as a hot solution, while the latter, which readily loses gas on warming, was added under cold conditions. Treated samples were stored at 0.5°C. unless otherwise stated. Direct

TABLE III. Effect of methyl bromide (MeBr) on growth

Culture no.	Genus	Culture (No MeBr)						5 cm. MeBr					
		1 day A* B*		2 days A	B	14 days A	B	1 day A	B	2 days A	B	14 days A	B
1	Micrococcus..............	+	−	+	−	+	+	+	−	+	−	+	−
2	"	+	+	+	+	+	+	+	−	+	−	+	+
3	Yeast...................	+	+	+	+	+	+	+	−	+	−	+	+
4	Micrococcus.	+	+	+	+	+	+	+	−	+	+	+	+
5	"	+	+	+	+	+	+	+	−	+	−	+	+
6	Flavobacterium..........	+	+	+	+	+	+	+	−	+ .	−	+	−
7	Micrococcus..............	+	+	+	+	+	+	+	+	+	+	+	+
8	"	+	−	+	−	+	+	+	−	+	−	+	−
9	Achromobacter...........	+	+	+	+	+	+	+	−	+	−	+	+
10	Micrococcus..............	+	−	+	−	+	+	+	−	+	−	+	−
11	"	+	+	+	+	+	+	+	−	+	+	+	+
12	Yeast...................	+	+	+	+	+	+	−	−	+	−	+	+
13	Micrococcus..............	+	−	+	−	+	+	+	−	+	−	+	+
14	Yeast...................	+	+	+ ˎ	+	+	+	+	−	+	−	+	−
15	"	+	+	+	+	+	+	+	−	+	−	+	+
16	Achromobacter...........	+	−	+	+	+	+	+	−	+	+	+	+
17	"	+	+	+	+	+	+	+	−	+	+	+	+
18	Micrococcus.............	+	−	+	−	+	+	+	−	+	−	+	+
19	Achromobacter...........	+	+	+	+	+	+	+	−	+	−	+	+
20	Micrococcus..............	+	−	+	−	+	+	+	−	+	−	+	+
21	Flavobacterium..........	+	−	+	−	+	+	−	−	+	−	+	+
22	Achromobacter...........	+	+	+	+	+	+	+	+	+	+	+	+
23	"	+	+	+	+	+	+	+	−	+	+	+	+
24	Micrococcus..............	+	+	+	+	+	+	+	−	+	−	+	+

*A = agar; B = broth; + = growth; − = no visible growth.

(Tarr 1943) or viable (Tarr and Bailey 1939) bacterial counts were made after a stated time.

Gas treatment of fish flesh was carried out as follows. Beakers containing the minced muscle were placed in a vacuum desiccator and subjected to a reduced pressure (3 or 6 cm. of mercury) for 2 minutes. The required amount (measured by the increase of the pressure) of the gas being studied was run in as quickly as possible, though in the case of gases which were very soluble in water (ethylene and propylene oxides) rapid solution of the gas in the muscle fluid made the

measurement of added gas only very approximate. The flesh was exposed to the gas for a given time, and then subjected to a reduced pressure (about 2 cm. of mercury) for 5 minutes in order to remove much of the gas from the fish. Air was then allowed to enter the desiccator, and the treated samples were stored at 0.5 or 10°C., direct bacterial counts being made at stated times.

The following compounds were studied in the experiments to be described: methyl formate, ethyl formate, methyl ether (purchased as 40 per cent solution in sulphuric acid) and propylene oxide (E.K. "Eastman"); ethylene glycol, pro-

of isolated bacterial cultures in broth and agar media.

10 cm. MeBr						25 cm. MeBr						50 cm. MeBr					
1 day		2 days		14 days		1 day		2 days		14 days		1 day		2 days		14 days	
A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	+	−	+	+	−	−	+	−	+	+	−	−	−	−	−	−
−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	+
−	−	+	+	+	+	−	−	+	−	+	+	−	−	−	−	−	+
−	−	+	−	+	+	−	−	+	−	+	−	−	−	−	−	+	−
−	−	+	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−
+	+	+	+	+	+	−	−	+	+	+	+	−	−	−	−	+	+
−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	−	+	+	−	−	−	−	−	+	−	−	−	−	−	−
−	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	−	+	+	−	−	−	−	+	+	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	−	+	+	−	−	−	−	−	+	−	−	−	−	−	+
−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	−	+	+	−	−	−	−	+	+	−	−	−	−	−	−
+	+	+	+	+	+	−	−	+	+	+	+	−	−	+	−	+	+
+	−	+	+	+	+	−	−	−	−	+	+	−	−	−	−	−	+
−	−	+	−	+	+	−	−	+	+	+	+	−	−	−	−	−	−

pylene glycol, trimethylene glycol, 1 : 4 dioxane, methyl bromide and ethylene dichloride (E.K. "Practical"); ethylene oxide, ethyl chloride and chloroform (E.K. "Technical"); Chloramine T (B.D.H.), Chloramine B (Solvay Process Co.); sodium chlorite (Mathieson Chemicals "Technical"), and sodium nitrite (B.D.H. "A.R."). A mixture of 5-m-dioxanol and 4-hydroxymethyl-1 : 3 dioxolane (isomeric glycerol formals) was kindly prepared by Dr. N. M. Carter of this Station. A sample of "Nipicide", a patent fish preservative, was kindly obtained by Mr. M. E. Stansby of the U.S. Fish and Wildlife Service. A rough analysis showed that it contained sodium benzoate and probably a chloramine.

COMPOUNDS APPLIED IN SOLUTION

The bacteriostatic action of most of the compounds listed above was studied in two tests, one using minced halibut muscle and 0.01 per cent of each compound, the other minced coho salmon (*Oncorhynchus kisutch*) muscle and 0.1 per cent, the treated samples being stored at 0.5°C. Direct bacterial counts were made on the samples at intervals.

The results (table IV) showed that none of the compounds studied exerted any important bacteriostatic effect in 0.01 per cent concentration. In 0.1 per

TABLE IV. Effect of various compounds on growth of bacteria in fish muscle.

Compound investigated	Experiment with halibut muscle using 0.1% of each compound		Experiment with coho salmon muscle using 0.1% of each compound
	Direct bacterial counts after		Direct bacterial counts after
	1 day	6 days	9 days
Controls (water)	2.5×10^6	2500×10^6	710×10^6
Ethylene glycol	2.1×10^6	2200×10^6	350×10^6
Propylene glycol	1.7×10^6	1400×10^6	1600×10^6
Trimethylene glycol	2.5×10^6	1100×10^6	1400×10^6
Glycerol formal	1.8×10^6	1200×10^6	2400×10^6
1 : 4 dioxane	3.2×10^6	2000×10^6	$460 \cdot \times 10^6$
Ethylene oxide	1.8×10^6	2600×10^6	2×10^6
Propylene oxide	......	......	55×10^6
Ethyl formate	......	......	160,000
Methyl formate	......	......	240,000
Chloroform	1.3×10^6	1200×10^6	2200×10^6
Chloramine B	1.5×10^6	1600×10^6	680×10^6
Chloramine T	2.1×10^6	1900×10^6	1300×10^6
"Nipicide"	1.5×10^6	1500×10^6	1000×10^6
Sodium benzoate	1.6×10^6	2100×10^6	440×10^6
Sodium chlorite	2.4×10^6	950×10^6	320×10^6
Sodium nitrite	1.7×10^6	1200×10^6	$28 \times 10^6*$
Ethylene dichloride	2.3×10^6	1900×10^6	....
Penicillic acid	......	......	110×10^6
4-Methoxy-2 : 5 toluquinone	......	......	300×10^6

*0.02% sodium nitrite used in this test.

cent concentration methyl formate, ethyl formate, ethylene oxide and propylene oxide strongly retarded bacterial multiplication. Penicillic acid and 4-methoxy-2 : 5 toluquinone exerted only feeble bacteriostatic action in this concentration. Sodium nitrite (0.02 per cent) was quite effective in this respect, but none of the other compounds studied caused any important inhibition of bacterial growth. Ethylene oxide, propylene oxide, penicillic acid and 4-methoxy-2 : 5 toluquinone caused a pronounced brownish discolouration and rancid odour in the salmon flesh, while methyl and ethyl formates caused an appearance similar to that assumed by flesh treated with weak acids.

Ethylene oxide and propylene oxide have found wide industrial use as insecticides, but in recent years several patents have been granted covering their application as bactericides. Thus Gross and Dixon (1937) patented the use of ethylene oxide and propylene oxide under reduced pressure for sterilizing (or partially sterilizing) a great variety of foods ranging from fresh meats to rolled oats, as well as soils, tobacco and surgical instruments. These writers assumed that ethylene glycol was formed in moist foods during treatment. Jensen (1943 unpub.) states that ethylene oxide must not be used for processing moist foods due to danger of ethylene glycol formation. As far as the writer is aware no published information is available concerning the amount of ethylene glycol formed in moist foods following ethylene oxide treatment. Wood (unpub.) found that he could obtain fish muscle which was bacteriologically sterile by treating it with ethylene oxide gas *in vacuo*. Moreover, he discovered that such treatment did not inactivate any of a number of its normal enzyme systems which he inves-

TABLE V. Effect of ethylene and propylene oxides on the rate of bacterial increase (shown as bacteria per gram by dried count) in fish muscle.

Applied in solution—halibut muscle—storage at 0.5°C.

	Control	Ethylene oxide (%)		Propylene oxide (%)	
		1	0 1	1	0 1
After 10 days	750×10⁶	300,000	440×10⁶	2 9×10⁶	720×10⁶

Applied as gases—lingcod muscle—storage at 10°C.

	Control	Ethylene oxide in cm.				Propylene oxide in cm.			
		5	10	20	30	5	10	20	30
After 4 days	*22,000×10⁶	670×10⁶	740,000	80,000	80,000	*9,000×10⁶	260×10⁶	30×10⁶	560,000
8 days		*	*	2,700×10⁶	720×10⁶		*	*	*6,300×10⁶

*Putrid

tigated. The possible value of this treatment as a method of rendering fish muscle free from bacteria for studies of its enzyme systems therefore is obvious. Ethylene oxide can be used successfully in commercial sterilization of comparatively dry substances such as spices (Griffith and Hall 1938, Baer 1941) and is very much more efficient than is ethylene glycol for this purpose (Yesair and Williams 1942).

The foregoing experiment showed that ethylene and propylene oxides in 0.1 per cent concentration successfully retarded multiplication of bacteria in the flesh of fatty fish (coho salmon) but also accelerated the development of rancidity. However, ethylene oxide in this concentration did not inhibit growth of bacteria in halibut flesh, and this result was verified in the present experiment in which it was found that 0.1 per cent of ethylene or propylene oxide did not occasion any marked reduction in the rate of bacterial increase in this fish, 1 per cent being required before a pronounced decrease in the number of bacteria was observed (table V).

Compounds Applied as Gases

Minced lingcod (*Ophiodon elongatus*) was exposed to reduced pressure (3 cm. of mercury), treated for 30 minutes with different partial pressures of *ethylene* and *propylene oxide* as previously described, and stored at 10°C. Direct bacterial counts were made at intervals, the results being given in table V. It will be seen that both gases effectively retarded bacterial multiplication in the flesh, ethylene oxide being more effective than propylene oxide.

Minced red cod (*Sebastodes* sp.) flesh was exposed to reduced pressure (6 cm. of mercury) and then different samples were exposed to different concentrations of *methyl bromide* in the usual manner. The following viable counts were obtained on the samples immediately after treatment with the partial pressures of methyl bromide given: Control 28,000; 5 cm., 27,000; 10 cm., 24,000; 25 cm , 23,000; 50 cm., 19,000. This showed that methyl bromide effected only a very slight decrease in the number of viable bacteria in the fish muscle. Subsequent storage of the treated samples at 0.5°C. showed that this gas treatment occasioned no noticeable improvement in keeping quality as judged by the odour and appearance of the samples. A similar experiment performed with flounder (*Lepidopsetta bilineata*) muscle yielded almost identical results.

Minced halibut flesh was subjected to a vacuum of 3 cm. of mercury and then different lots were exposed to 30 cm. partial pressure on each of *methyl ether, ethyl ether, methyl formate* and *ethyl chloride* for 10 minutes. The direct bacterial counts after 6 days were as follows: Control (not gassed) 4,300 $\times 10^6$; methyl ether, 1,100 $\times 10^6$; ethyl ether, 1,500 $\times 10^6$; methyl formate, 110,000; and ethyl chloride, 1,500 $\times 10^6$. These results show that only methyl formate caused a marked decrease in bacterial content, the other gases having only a very minor inhibitory effect. The flesh treated with methyl formate assumed a whitish "coagulated" appearance similar to that exhibited by flesh treated with acid solutions.

DISCUSSION

The fact that the glycols studied did not prevent the multiplication of bacteria in fish flesh shows that the powerful anti-bacterial action of ethylene and propylene oxide cannot be attributed to the corresponding glycols which might be formed on hydrolysis. In this connection it must be noted that, while minute traces of the vapours or "aerosols" of some glycols are particularly toxic to certain bacteria and viruses, their solutions when in direct contact with the organisms are usually quite ineffective (Robertson, Brigg, Puck and Miller 1942; Robertson, Puck, Lemon and Loosli 1943). It is of interest that, while methyl bromide readily destroyed fish spoilage bacteria in ordinary nutrient medium, it had practically no effect on those naturally present in fish muscle. The reason for this is not clear, but it is suggested that, since methyl bromide is almost insoluble in water and highly soluble in fats, it was rapidly taken up by the latter and did not reach the organisms in lethal concentration.

It would seem that a rather high concentration of ethylene oxide or propylene oxide is required to inactivate bacteria. Thus from 0.1 to 1.0 per cent was found

necessary to cause marked reduction of viable bacteria in fish muscle, the lower concentration being sufficient in the case of fatty fish (salmon) but not adequate for lingcod. Probably the activity of these compounds when applied in gaseous form is due to their very high solubility in water, which may mean that, under the conditions of the experiments recorded in this paper, over 1 per cent of the oxide was in some cases in solution in the fish muscle fluids and in intimate contact with the bacteria. Both methyl and ethyl formate appeared to be as active in destroying bacteria in fish flesh as ethylene oxide, but caused an unnatural "coagulated" appearance in treated flesh.

With the possible exception of sodium nitrite which is probably innocuous if properly used (Tarr and Carter 1942), none of the compounds studied would seem of much value as preservatives for fresh fish. Though ethylene oxide proved a very efficient bactericide its use is not indicated because of the possibility that ethylene glycol might be formed. Propylene oxide was quite effective but it was found very difficult to remove from fish flesh, and flesh treated with it assumed a curious flavour. In this connection it must be noted that Sudendorf and Kröger (1931) found that ethylene oxide was rather slowly removed from various treated foods by ordinary aeration. It is probable that it would be much more readily removed by aeration under partial vacuum. Methyl and ethyl formates destroyed the bacteria in fish flesh very efficiently but adversely affected its appearance. Penicillic acid and 4-methoxy-2 : 5 toluquinone in 0.1 per cent concentration were not so effective as 0.02 per cent sodium nitrite and accelerated rancidity in the muscle of fatty fish.

SUMMARY

Penicillic acid and 4-methoxy-2 : 5 toluquinone in 0.01 or 0.001 per cent concentration inhibited growth of pure cultures of certain fish spoilage bacteria on laboratory media, their action being more pronounced in slightly acid than in neutral or faintly alkaline media. Neither compound in 0.01 per cent concentration was effective in retarding bacterial spoilage of halibut muscle, and in 0.1 per cent concentration retarded bacterial spoilage of coho salmon muscle only feebly.

Isolated cultures of fish spoilage bacteria on laboratory media were rapidly inactivated by treatment with methyl bromide, but treatment of fish muscle with different partial pressures of this gas under partial vacuum occasioned only a very minor reduction in its viable bacterial content.

Treatment of fish muscle with ethylene oxide, propylene oxide and methyl formate as gases under partial vacuum markedly delayed the increase of bacteria therein. When applied in solution, from 0.1 to 1.0 per cent of these compounds was required in order to markedly delay bacterial growth in fish muscle. Ethyl formate appeared to be as active as methyl formate in preventing bacterial growth, but both compounds occasioned a very marked "coagulated" appearance in treated fish flesh.

Sodium nitrite (0.02 per cent) retarded bacterial increase in fish flesh, but penicillic acid, 4-methoxy-2 : 5 toluquinone, sodium chlorite, sodium benzoate

and a patent fish preservative studied were much less effective in this respect. None of a number of other compounds studied delayed bacterial growth appreciably in fish flesh.

ACKNOWLEDGMENTS

REFERENCES

ASHLEY, J. N. *J. Chem. Soc.*, **1937**, 1471-1472, 1937.

BAER, J. M. U.S. Patent No. 2,229,360, 1941.

BIRKINSHAW, J. H., A. E. OXFORD AND H. RAISTRICK. *Biochem. J.*, **30**, 394-411, 1936.

DUDLEY, H. C., AND P. A. NEAL. *Food Res.*, **7**, 421-429, 1942.

GRIFFITH, C. L., AND L. A. HALL. U.S. Patent No. 2,107,697, 1938.

GROSS, P. M., AND L. F. DIXON. U.S. Patent No. 2,075,845, 1937.

MRAK, E. M. *Rep. Ann. Date Grower's Inst.*, **18**, 3-4, 1941. (Through *Chem. Abst.*, **36**, 3863, 1942).

OXFORD, A. E., H. RAISTRICK AND G. SMITH. *Chem. and Ind.*, **61**, 22-24, 1942.

ROBERTSON, O. H., E. BRIGG, T. T. PUCK AND B. F. MILLER. *J. Exp. Med.*, **75**, 593-610, 1942.

ROBERTSON, O. H., T. T. PUCK, H. F. LEMON AND C. G. LOOSLI. *Science*, **97**, 142-144, 1943.

SUDENDORF, T., AND E. KRÖGER. *Chemiker-Ztg.*, **55**, 549-550, 570-572, 1931.

TARR, H. L. A. *J. Fish. Res. Bd. Can.*, **4**, 367-377, 1940.

    J. Fish. Res. Bd. Can., **5**, 265-275, 1941.

    J. Fish. Res. Bd. Can., **6**, 74-89, 1942.

    J. Fish. Res. Bd. Can., **6**, 119-128, 1943.

TARR, H. L. A., AND B. E. BAILEY. *J. Fish. Res. Bd. Can.*, **4**, 327-336, 1939.

TARR, H. L. A., AND N. M. CARTER. *J. Fish. Res. Bd. Can.*, **6**, 63-73, 1942.

TARR, H. L. A., AND P. A. SUNDERLAND. *Fish. Res. Bd. Can. Prog. Rep. Pac.*, **39**, 13-16, 1939.

    J. Fish. Res. Bd. Can., **5**, 148-163, 1940.

YESAIR, J., AND O. B. WILLIAMS. *Food Res.*, **7**, 118-126, 1942.

DRIED WHOLE EGG POWDER

XIII. EFFECT OF HEAT TREATMENT ON COLOUR[1]

BY W. HAROLD WHITE[2] AND G. A. GRANT[3]

Abstract

Egg powder from two Canadian plants was heated at temperatures from 26.7° to 60.0° C. (80° to 140° F.). Samples were removed for quantitative colour measurements after periods of three hours to seven days. Initially the powders from the two sources differed significantly with respect to both brightness and colour quality. However, their behaviour with heat treatment was essentially similar. Little change in either total intensity or colour quality was noted at temperatures below 35.0° C. (95° F.). Above 35° C. appreciable changes in both brightness and colour quality occurred; the magnitude of these changes increased with increase in temperature and time of treatment. The total intensity decreased, indicating a general darkening of the powder. The amount of light scattered in the green region of the spectrum decreased, while that in the red increased. Some change was also observed in a portion of the violet region.

Introduction

Certain observations have indicated that Canadian dried egg powders are lighter in colour than those from other sources. Such differences presumably are due at least in part to variations in the colour of the liquid egg as a result of different feeding practices in different countries. However, there is little doubt that manufacturing conditions, including time and temperature factors, also exert some influence on the colour of the product. Furthermore, it was suspected that light-coloured powders reflected minor changes more readily than darker powders from other countries. Since temperature at all stages of production and handling has been shown to be an important factor affecting quality in other respects (1, 2, 3), the present study was undertaken to assess the effect of heat treatment on the colour of egg powder.

Samples of egg powder may differ from one another with respect to either or both of two attributes of colour, namely total intensity or brightness, and chroma or colour quality. Brightness is determined by the ability of the sample to scatter all components present in the incident light. Variations in hue, on the other hand, arise from differential scattering of the incident light in one or more wave bands by individual samples.

[2] *Formerly Biochemist, Food Investigations; now Director of Research F. W. Horner Ltd., Montreal, Que.*

[3] *Laboratory Assistant, Food Investigations.*

Materials and Procedure

As described in an earlier paper of this series (2), egg powders collected from two Canadian plants were heated rapidly to and maintained at 26.7°, 35.0°, 43.3°, 51.7°, and 60.0° C. (80°, 95°, 110°, 125°, and 140° F.). Samples were removed for colour measurement after periods of 3, 6, and 12 hr.; and 1, 2, 3, 4, 5, 6, and 7 days. These conditions were chosen with the primary object of assessing the effect of various cooling practices on several attributes of quality including colour.

Objective colour measurements were made with a photo-electric colour comparator developed previously in these laboratories for use on meat (4, 5). This instrument permitted measurement both of the total amount of light scattered by egg powder and of that scattered in each of nine bands of the visible spectrum.

Results

Colour Intensity or Total Brightness

The results are shown in Tables I and II, and in Fig. 1. Because of the large amount of data and the relatively minor differences in brightness, statistical treatment of the results was necessary. An analysis of variance (Table I) showed that differences in temperature, time of heating, and the

TABLE I

ANALYSIS OF VARIANCE FOR THE EFFECT OF VARIOUS HEAT TREATMENrS ON THE
BRIGHTNESS OF DRIED EGG POWDERS SECURED FROM TWO CANADIAN PLANTS

Variance attributable to:	D.f.	Mean square
Temperature	4	942.1**
Time	9	170.1**
Plants	1	2275**
Temperature × time	36	44.4**
Plants × temperature	4	3.422**
Plants × time	9	1.097
Plants × temperature × time	36	0.5893
Duplicate error	100	0.1729

** *Indicates 1% level of statistical significance.*

source of the powder all had statistically significant effects on the colour intensity or brightness of the powder. The magnitude and direction of these changes are shown in Table II. The mean brightness, as averaged over all other conditions, decreased with increased temperature or time of heating; these differences were greatest between the three highest temperatures, and during the first three days of heating. The powder from Plant I was brighter than that from Plant II.

The results are shown in greater detail in Fig. 1. In general there was little change at the lowest temperature and progressively greater decreases with time at the higher temperatures. It is of importance to note that the
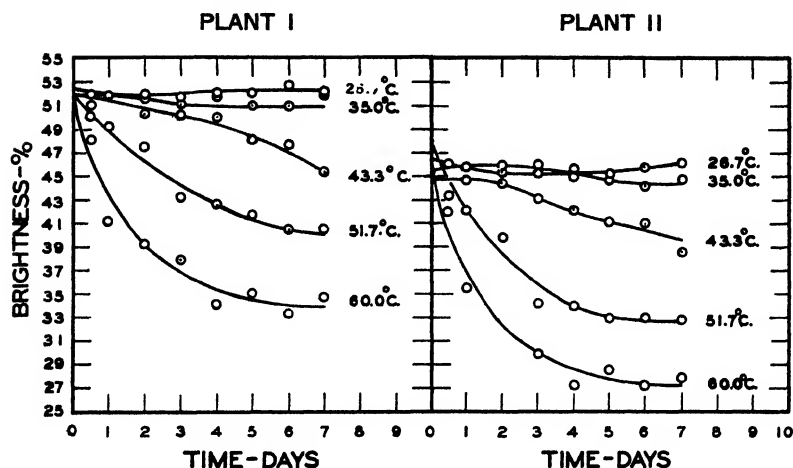
FIG. 1. *Effect of various heat treatments on the colour intensity or total brightness of egg powders from two Canadian plants.*

TABLE II

EFFECT OF VARIOUS HEAT TREATMENTS ON THE MEAN VALUES OF THE BRIGHTNESS
OF DRIED EGG POWDERS SECURED FROM TWO CANADIAN PLANTS

Factor	Mean brightness[1]	Necessary difference[2]
Temperature, °C.		
26.7	48.9	
35.0	48.4	
43.3	46.7	0.35
51.7	42.2	
60.0	37.4	
Time, days		
0.13	49.2	
0.25	48.4	
0.50	47.7	
1	46.1	
2	44.8	0.50
3	43.3	
4	42.5	
5	42.1	
6	41.7	
7	41.5	
Plant		
I	48.1	
II	41.4	

[1] *Mean brightness for all other conditions over the whole experiment.*
[2] *Necessary difference required to exceed 5% level of statistical significance.*

behaviour of powders from different sources was essentially similar. Differences between powders from the two plants were approximately of the same

magnitude at all temperatures, while similarity of behaviour was even more noticeable for the differential effects of plants with time. Thus, the darker powder from Plant II exhibited the same general behaviour under the conditions studied as the lighter powder from Plant I.

The average differences between powders exposed to the minimum and maximum conditions of temperature and of time of treatment were not much greater than the average difference between powders from the two sources (Fig. 1 and Table II). From this it may be inferred either that factors associated with plant practice other than the rate of cooling, possibly the conditions of drying, have an important influence on the brightness of egg powder or that the initial liquid egg differed in some manner affecting colour intensity.

Chroma or Colour Quality

While differences between powders from the two sources and changes induced by heat treatment were evident in certain colour bands (Table III) their magnitudes were usually small, thus requiring analyses of variance to assess their significance (Table IV). Fig. 2 shows these changes more clearly with respect to differences between plants and between extremes of time and

TABLE III

EFFECT OF VARIOUS HEAT TREATMENTS ON THE MEAN VALUES OF THE COLOUR OF DRIED EGG POWDERS SECURED FROM TWO CANADIAN PLANTS

Factor studied	Mean scatter[1], %								
	Band 1	Band 2	Band 3	Band 4	Band 5	Band 6	Band 7	Band 8	Band 9
	3850 – 4340 Å	4340 – 4580 Å	4580 – 4870 Å	4870 – 5250 Å	5250 – 5560 Å	5560 – 5840 Å	5840 – 6140 Å	6140 – 6440 Å	Above 6440 Å
Temperature, °C.									
26.7	3.63	6.52	8.57	15.0	15.1	25.3	11.7	7.00	7.18
35.0	3.52	6.62	8.59	15.0	15.0	25.4	11.7	7.07	7.20
43.3	3.33	6.68	8.72	15.1	14.8	25.2	11.8	7.08	7.30
51.7	2.93	6.60	8.79	14.7	14.3	25.4	12.1	7.38	7.71
60.0	2.81	6.71	8.79	14.4	13.8	25.4	12.3	7.59	8.16
Time, days									
0.13	3.34	6.69	8.47	15.0	15.2	25.1	11.9	7.06	7.23
0.25	3.53	6.56	8.56	14.9	15.0	25.5	11.8	7.07	7.05
0.50	3.55	6.74	8.55	15.0	14.9	25.3	11.8	7.00	7.22
1	3.49	6.55	8.68	15.0	14.7	25.4	11.9	7.12	7.35
2	2.92	6.68	8.69	14.8	14.7	25.5	12.0	7.29	7.63
3	3.22	6.45	8.84	15.0	14.4	25.3	12.0	7.30	7.62
4	3.33	6.42	8.79	14.6	14.5	25.3	12.0	7.35	7.71
5	3.18	6.80	8.75	14.7	14.3	25.3	12.0	7.29	7.82
6	3.02	6.78	8.84	14.7	14.3	25.2	12.1	7.39	7.81
7	2.88	6.71	8.82	14.8	14.2	25.4	12.1	7.37	7.67
Plant									
I	3.34	6.73	8.80	15.0	14.9	25.3	11.7	7.05	7.16
II	3.14	6.53	8.58	14.6	14.3	25.4	12.2	7.39	7.86

Mean scatter for all other conditions over the whole experiment.

temperature. Egg powder from Plant II scattered less light in the violet and more in the red regions of the spectrum than that from Plant I. It will be

TABLE IV

ANALYSES OF VARIANCE FOR THE EFFECT OF VARIOUS HEAT TREATMENTS ON THE COLOUR OF DRIED EGG POWDERS SECURED FROM TWO CANADIAN PLANTS

Variance attributable to:	Degrees of freedom	Mean square								
		Band 1	Band 2	Band 3	Band 4	Band 5	Band 6	Band 7	Band 8	Band 9
		3850 – 4340 Å	4340 – 4580 Å	4580 – 4870 Å	4870 – 5250 Å	5250 – 5560 Å	5560 – 5840 Å	5840 – 6140 Å	6140 – 6440 Å	Above 6440 Å
Temperature	4	5.1**	0.17	0.45	3.3**	10.7**	0.47*	2.8**	2.5**	7.1**
Time	9	1.2**	0.33	0.36	0.48*	2.4**	0.27	0.25*	0.42**	1.5**
Plants	1	2.0*	2.1**	2.3**	7.9**	14.4**	0.52	9.1**	5.9**	24.4**
Temperature × time	36	0.39	0.38	0.26	0.49**	0.54	0.20	0.21*	0.16**	0.30**
Plants × temperature	4	0.31	0.09	0.28	0.21	0.51	0.08	0.17	0.16*	0.08
Plants × time	9	0.54	0.40	0.16	0.40	0.13	0.13	0.06	0.10	0.13
Plants × time × temperature	36	0.38	0.28	0.22	0.20	0.34	0.14	0.11	0.06	0.06
Duplicate error	100	0.14	0.13	0.11	0.13	0.08	0.11	0.06	0.05	0.08

* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% level of statistical significance.*
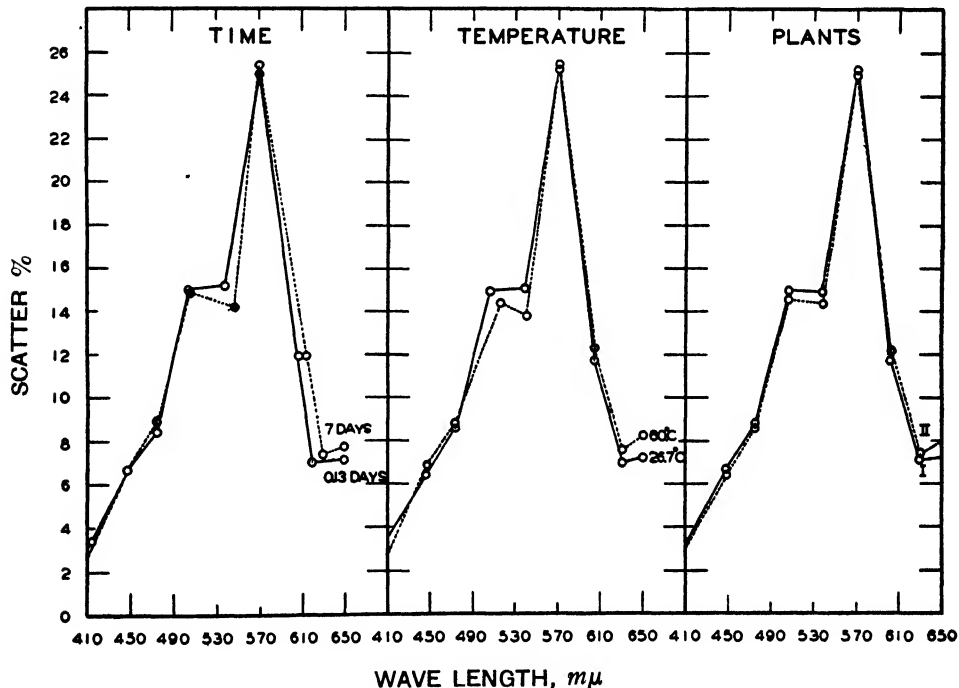
FIG. 2. *Effect of source and of period and temperature of heat treatment on the colour quality of egg powder.*

remembered that the powder from Plant II was generally darker, as shown by brightness measurements (Table II).

From Table IV it may be seen that the source and the temperature and period of treatment each had statistically significant effects on the colour in all bands except 2, 3, and 6. The greatest changes occurred in Bands 1, 4 and 5, and 7 to 9. It may be noted here that the values for Band 1 show that a definite decrease occurred in the amount of light scattered in a portion of the violet spectral region. However, such changes have little practical significance since visual acuity is low in this region. Bands 5 (5250 to 5560 Å) and 8 (6140 to 6440 Å) were chosen for more detailed presentation as typical of regions in which larger and more important changes occurred.
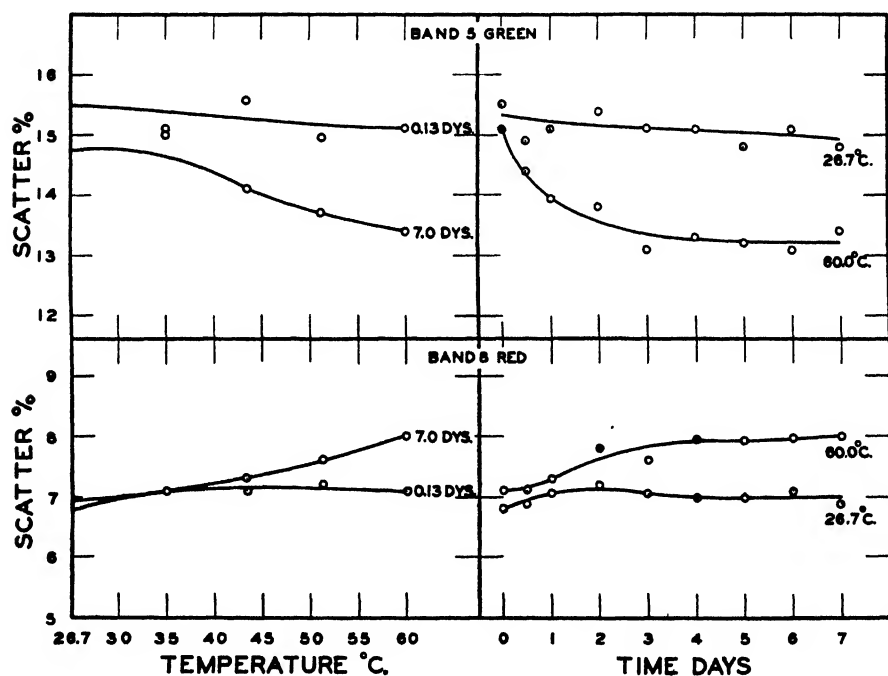
FIG. 3.   *Effect of the temperature and period of heat treatment on the colour of egg powder in the spectral regions of 5250 to 5560 Å and 6140 to 6440 Å.*

Fig. 3 shows the changes in Bands 5 and 8 at selected times and temperatures.   In terms of colour these changes correspond to a reduction in the green component and an increase in red scatter, in response to increasing time and temperature. Visually, the typical yellow colour of egg powder decreased, and the brown colour increased with increasing severity of heat treatment.

In both Bands 5 and 8, as shown by Fig. 3, the colour changes at the maximum temperature studied appear to be approaching equilibrium before the end of the period of treatment was reached.

Conclusions

From the foregoing results it is apparent that, under adverse time and temperature of storage, the colour of egg powder is affected with respect to both intensity and colour quality. The powders darkened, accompanied by a decrease in the green portion of the spectrum, and an enhancement of the red scatter. Above 35° C., these adverse colour changes assumed more serious proportions as time and temperature of treatment were increased.

No comment is offered on the mechanism of the changes in colour quality; though the fact that three separate colour bands were affected suggests that specific pigments were involved in these changes.

Acknowledgments ·

The authors wish to thank Mr. M. W. Thistle for assistance in the preparation of the manuscript, and Mr. W. D. B. Reid for making the statistical computations.

References

1. Gibbons, N. E. and Fulton, C. O.   Can. J. Research, D, 21 : 332-339.   1943.
2. White, W. H. and Thistle, M. W.   Can. J. Research, D, 21 : 194-202.   1943.
3. White, W. H., Thistle, M. W., and Reid, M.   Can. J. Research, D, 21 : 271-276.   1943.
4. Winkler, C. A., Cook, W. H., and Rooke, E. A.   Can. J. Research, D, 18 : 435-441.   1940.
5. Woodcock, A. H.   Can. J. Research, D, 21 : 90-97.   1943.

FLUORESCENCE DEVELOPMENT IN VARIOUS FOOD PRODUCTS[1]

By Jesse A. Pearce[2]

Abstract

Fluorescing substances developed in the following materials during storage: high protein foods, represented by dried whole milk powder, dehydrated pork, and soya flour; high carbohydrate foods, represented by dried banana flakes and dried parsnips; and a mixed foodstuff, represented by ration biscuits.

The only change occurring in stored shortenings was a decrease of fluorescing substances in hydrogenated linseed oils. Serum extracted from rancid butter had a higher fluorescence value than serum from fresh butter. In substances containing a high proportion of fat, fluorescence values bore little relation to deterioration as assessed by peroxide oxygen determinations.

Fluorescence tests were unsatisfactory for dried milk powders and soya flour. However, they may prove useful as a measure of quality for dehydrated pork, dried banana, dried parsnips, ration biscuits, and butter. Fluorescence measurements may also detect reversion in hydrogenated linseed oil shortenings.

Introduction

The ultimate criterion of quality in a foodstuff is its acceptability when consumed and for this reason many investigations involving food quality require panels of selected persons trained to taste the material and estimate its quality. This subjective procedure entails various errors, e.g., when samples are numerous the tasters may become bored and careless long before the conclusion of the experiment. Therefore, the use of objective tests that do not vary with time is highly desirable.

A fluorescence measurement proved to be a useful measure of the quality of dried whole egg powder (6, 8), and also gave an indication of the storage history of wheat germ (2). Further investigation indicated that protein deterioration contributed to fluorescence development in dried egg powder (3). It seemed of interest, therefore, to measure fluorescence changes during the storage of high protein and other foodstuffs. The high protein foods selected for this survey were dried whole milk powder, dehydrated pork, and soya flour; the high carbohydrate foods, dried banana flakes and dried parsnips; and 12 shortenings were used as representing fatty foodstuffs. Two types of ration biscuits were also investigated, since they are essentially a mixture of each of the three foregoing classes of foodstuffs.

Work on dried eggs had shown that it was possible to have high fluorescence values and poor quality in the product, without the appearance of fat deterioration as assessed by peroxide oxygen determinations (9). Therefore, peroxide oxygen measurements were included in quality tests made on foods containing appreciable quantities of fat.

---

[1] *Manuscript received March 20, 1944.*

[2] *Biochemist, Food Investigations.*

Materials and Methods

The products selected were from a variety of sources. The dried whole milk used consisted of drum- and spray-dried powders from one company and a spray-dried sample from another company. The dehydrated pork was prepared in these laboratories. The soya flour was a commercially available full fat product. The high carbohydrate foods (dried banana and dried parsnips) were also available commercially.

The shortenings studied were of the following types: hydrogenated vegetable, from two sources; stabilized hydrogenated vegetable, from three sources; compound vegetable, compound vegetable containing 0.05% of a wheat germ oil antioxidant,* and a stabilized compound vegetable; mixed animal and vegetable, from two sources; and a hydrogenated linseed oil.

The biscuits used were from two companies; one contained soya flour as a source of protein, the other, dried milk powder.

For dried milk powder and emergency ration biscuits, the storage temperatures used were 23.9°, 32.2°, and 47.8° C. (75°, 90°, and 118° F.); and for soya flour, dried banana, and dried parsnips, 23.9°, 37.8°, and 47.8° C. (75°, 100°, and 118° F.). The storage temperatures for dehydrated pork were 23.9° and 37.8° C. (75° and 100° F.) and for the shortenings, 37.8° C. (100° F.) only. All samples were stored in air in closed containers to prevent moisture changes.

The methods of measuring fluorescence were somewhat similar to those described for dried egg powder (6, 7). For example, 1 gm. of defatted milk powder was extracted with a 10% potassium chloride solution and made up to 250 ml. to bring the reading within the range of the scale on the Coleman photofluorometer. Dried bananas and dried parsnips were not defatted prior to extraction with the protein solvent. The shortenings were dissolved in petrol ether (1 gm. in 50 ml.) and the fluorescence of the resulting solution determined. The Coleman photofluorometer was standardized with quinine sulphate solutions as described for dried whole egg powder (6). The fluorescence readings are recorded in photofluorometer units.

Palatability scores were determined for reconstituted dried milk and reconstituted dried pork by panels of 14 tasters. The ratings applied were based on scores from 10 to 0; 10 corresponding to an excellent fresh product. Rancidity in the shortenings was assessed by a panel of four persons who smelled each sample and scored it as rancid or sweet. Dried bananas, dried parsnips, and soya flour were not tested organoleptically.

Peroxide oxygen determinations were made on foodstuffs in which the fat content was appreciable. The method used was similar to that described for pork fat (10). Peroxide oxygen values are recorded as ml. 0.002 N thiosulphate per gram of fat.

* *Formula C, Viobin (Canada) Ltd., Montreal.*

Results

High Protein Foodstuffs

Changes in organoleptic score and fluorescence values of stored milk powders are shown in Fig. 1. Fluorescence measurements were less satisfactory for milk powders from the Company X. The fluorescence value of both drum- and spray-dried powders from this company remained constant for about eight weeks and then increased, the increase being most rapid at the highest tem-
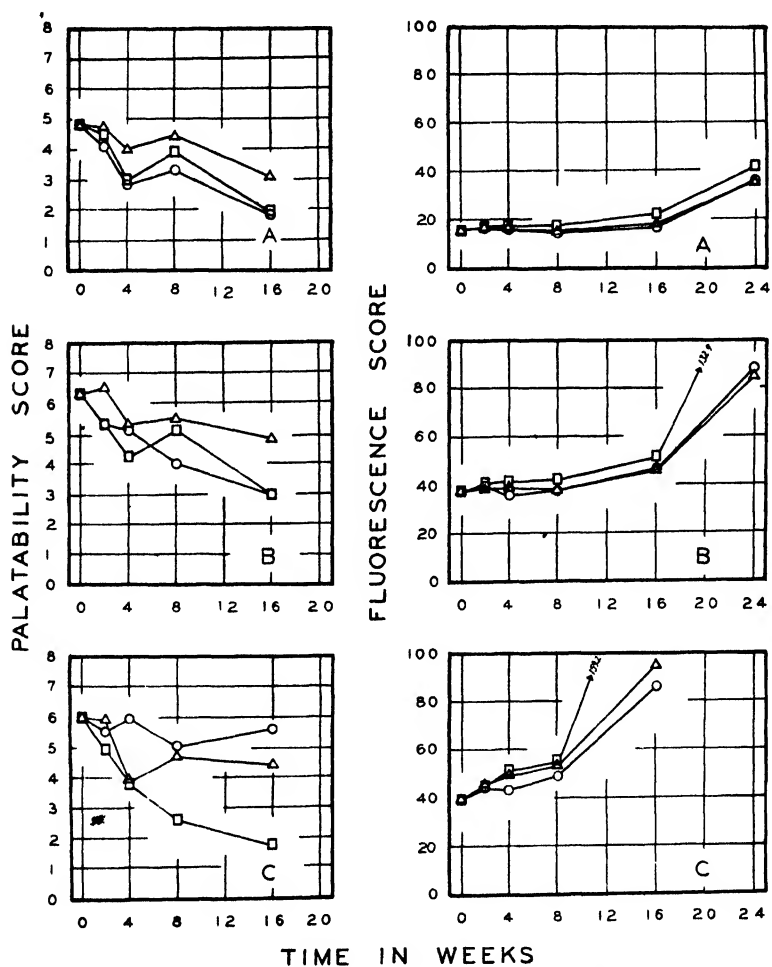
FIG. 1. *Palatability and fluorescence changes occurring in stored milk powders: A—drum-dried, Company X; B—spray-dried, Company X; C—spray-dried, Company Y. Storage temperatures:* □ *47.8° C. (118° F.);* △ *32.2° C. (90° F.);* ○ *23.9° C. (75° F.).*

perature. The low fluorescence values of the drum-dried powder is probably a result of protein coagulation occurring during this drying process (11). For these powders there was no significant association between palatability and fluorescence values, but the over-all linear correlation for spray-dried

powder from Company Y was $-0.950**$. It is unlikely that fluorescence measurements will be of value in predicting milk powder quality although it is interesting to note that fluorescing substances increase on storage.

The results of peroxide oxygen measurements on fat extracted from the stored milk powders are given in Table I. Peroxide oxygen increase did not

TABLE I

PEROXIDE OXYGEN VALUES (AS ML. 0.002 N THIOSULPHATE PER GM. OF EXTRACTED FAT) IN STORED WHOLE MILK POWDERS

Material	Storage temperature		Peroxide oxygen value after storage for		
	°C.	°F.	8 wk.	16 wk.	24 wk.
Drum-dried—Company X	23.9	75	5.8	19.7	18.4
	32.2	90	0	0	11.0
	47.8	118	0	0	19.5
Spray-dried—Company X	23.9	75	1.6	6.4	18.8
	32.2	90	0	3.2	14.7
	47.8	118	0	12.8	30.8
Spray-dried—Company Y	23.9	75	0	18.9	43.0
	32.2	90	0	0	22.4
	47.8	118	0	55 5	37.8

occur in any of the powders until after eight weeks' storage and bore no relation to fluorescence development. Peroxide oxygen values indicated a peculiar phenomenon occurring in dried milk powders, i.e. the peroxide oxygen values were much smaller at $32.2°$ C. ($90°$ F.) than at the other two temperatures. Also, samples from Company X stored at $32.2°$ C. ($90°$ F.) had higher palatability scores than those stored at $23.9°$ and $47.8°$ C. ($75°$ and $118°$ F.). This phenomenon is receiving further attention.

Fluorescence determinations were made on dehydrated pork, prepared in these laboratories (4). It was observed that samples subjected to prolonged drying periods had fluorescence readings of 30 to 70 photofluorometer units while those dried more quickly had values of 15 to 20 photofluorometer units. These results suggested that dehydrated pork would develop fluorescing substances as deterioration proceeded. Fig. 2 shows the results of measurements on stored dehydrated pork. Fluorescing substances were formed during storage and in greater quantities at the higher temperature. A linear correlation ($r = -0.794**$) was observed between fluorescence value and palatability score; it is therefore possible that fluorescence values may prove useful as a measure of quality of dehydrated pork.

Peroxide oxygen development in this material exhibited unexpected behaviour. High values were reached after a short period in storage. These

** *Exceeds 1% level of statistical significance.*

values exceeded the level generally associated with rancidity in cured or fresh pork during chill or frozen storage (1), but were not associated with any noticeable off-flavour in the dehydrated product. Again, no relation between peroxide oxygen values and fluorescence values was evident.
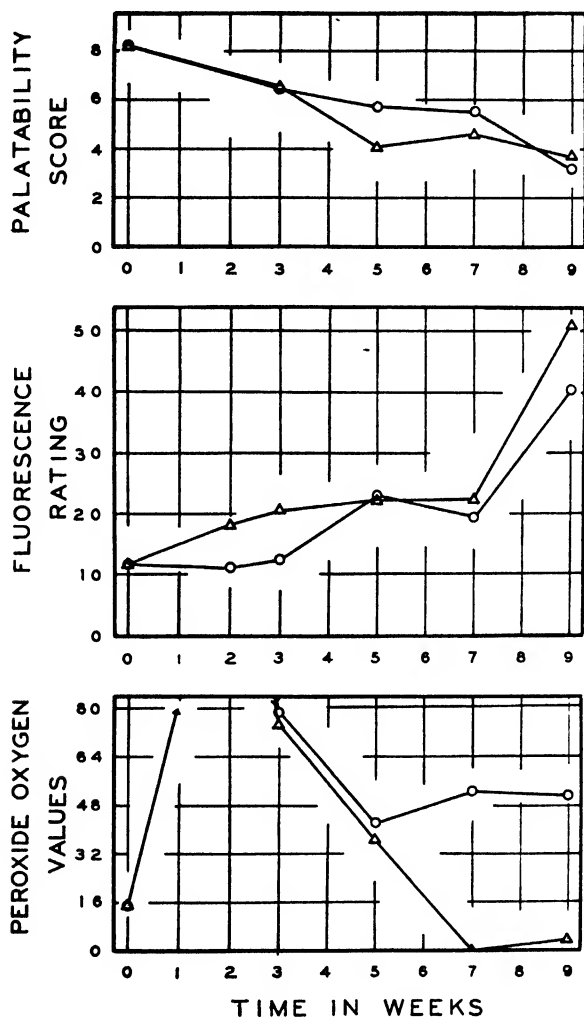
FIG. 2. *Palatability, fluorescence, and peroxide oxygen changes occurring in stored dehydrated pork. Storage temperatures:* $\triangle$ 37.8° C. (100° F.); $\bigcirc$ 23.9° C. (75° F.).

Soya flour was the only material studied in which fluorescence values developed almost uniformly at the three temperatures studied (Fig. 3). Therefore while fluorescence development occurs it is unlikely that this measurement will be of value in predicting quality, unless temperature effects are of no consideration. The peroxide oxygen value of soya flour after 15 weeks' storage at 47.7° C. (118° F.) was 0.9 ml., all other samples having zero values.

While fluorescence development occurred in each of the high protein foods studied, only for dehydrated pork did it appear likely that fluorescence would prove useful as a measure of quality. There appeared to be no relation between fluorescence development and peroxide oxygen changes in the fatty fraction of these foodstuffs.
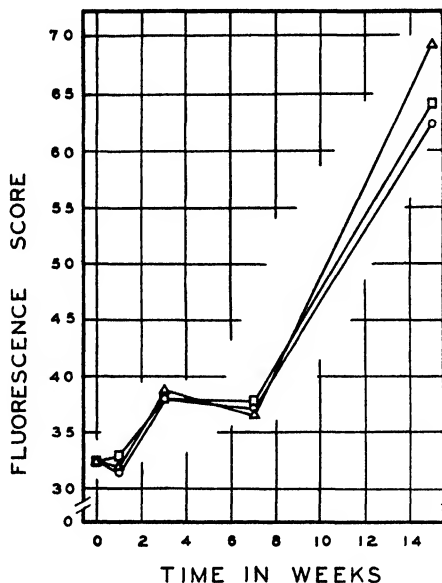
FIG. 3. *Fluorescence changes occurring in stored soya flour. Storage temperatures:* □ 47.8° C. (118° F.); △ 37.8° C. (100° F.); ○ 23 9° C. (75° F.).

High Carbohydrate Foods

In both dried bananas and dried parsnips (Fig. 4), fluorescing materials increased during storage, and the rate of increase in fluorescing substances was more rapid as the temperature was increased. In both cases fluorescence increase was accompanied by noticeable browning of the stored material, the browning being more pronounced at the higher storage temperatures. While no other measurements were made on these materials, it seems likely that fluorescence increase might be a test of quality of stored carbohydrate foodstuffs.

Fats

For most of the shortenings studied, any change in fluorescence value occurring during 15 weeks' storage at 37.8° C. (100° F.) was smaller than the variability in the determination with the single exception of hydrogenated linseed oil (Table II). In this material fluorescence decreased with increased storage time. Reversion to the odour of linseed oil had begun in the hydrogenated linseed oil after only one week's storage and increased in intensity with increased storage time. It may be that this can be followed objectively by measuring the decrease in fluorescing substances.
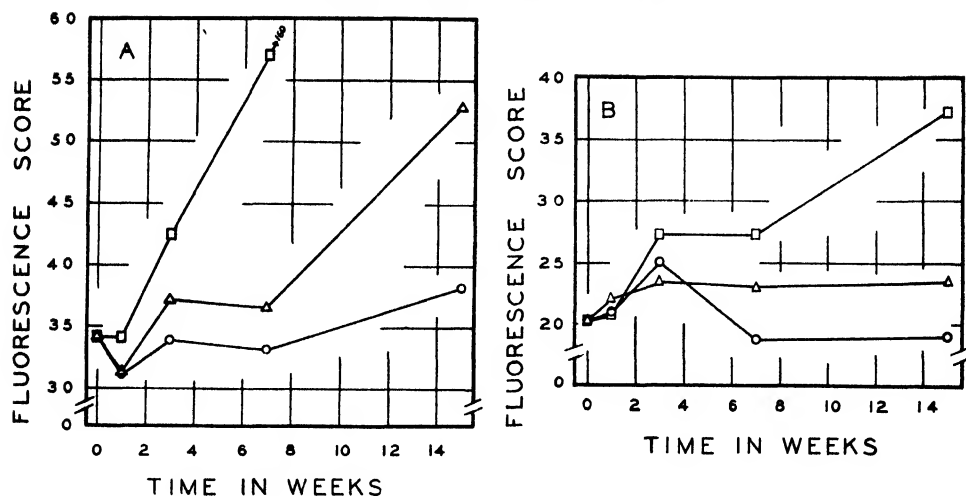
FIG. 4. *Fluorescence changes occurring in stored dried banana flakes (A) and dried parsnips (B). Storage temperatures:* □ *47.8° C. (118° F.);* △ *37.8° C. (100° F.);* ○ *23.9° C. (75° F.).*

TABLE II

FLUORESCENCE CHANGES OCCURRING IN FATS STORED AT 37.8° C. (100° F.) COMPARED WITH
RANCIDITY DEVELOPMENT

Type of fat	Fluorescence values		Peroxide oxygen value after 15 weeks*	Rancidity detected organolept- ically after:
	Initial	After 15 wk.		
Hydrogenated linseed oil	67.4	52.6	0	—**
Hydrogenated vegetable	60.0	60.5	9.0	15 wk.
Hydrogenated vegetable	40.2	38.7	27.0	15 wk.
Hydrogenated vegetable, stabilized	40.2	42.8	5.6	
Hydrogenated vegetable, stabilized	38.6	38.2	0	
Hydrogenated vegetable, stabilized	60.0	61.4	6.1	
Compound hydrogenated vegetable	44.8	43.6	22.5	15 wk.
Compound hydrogenated vegetable, with Formula *C*	44.1	41.7	67.4	15 wk.
Compound hydrogenated vegetable, stabilized	36.3	38.1	14.9	
Mixed animal and hydrogenated vegetable	48.8	48.1	(9.2)*** 13.1	7 wk.
Mixed animal and hydrogenated vegetable	38.6	34.8	13.8	
Lard	28.0	30.2	(22.8)*** 38.5	7 wk.

* *Initial peroxide oxygen value zero ml. 0.002 N thiosulphate per gm. of fat in all cases.*
** *Linseed oil reversion noticeable after one week's storage.*
*** *Values in parentheses for seven week period when rancidity first detected organoleptically.*

A further possibility of applying fluorescence measurements was indicated in the course of work on fresh and rancid butter. Butter serum diluted in the proportion of 1 ml. of serum to 20 ml. of 10% potassium chloride solution gave the following fluorescence readings: fresh butter, 28.2; rancid

butter, 60.0*. It must be remembered, however, that butter serum would probably contain only protein material and water-soluble breakdown products of the fats.

Ration Biscuits

As mentioned previously biscuits were selected as combining each of the three types of foodstuffs considered above. Only fluorescence measurements were made on these materials; the results are shown in Fig. 5. There are
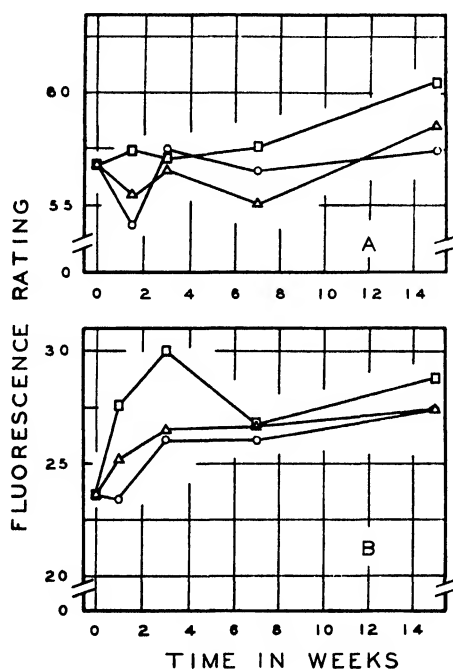
FIG. 5. *Fluorescence changes occurring in stored emergency ration biscuits. A—contains soya flour; B—contains milk powder. Storage temperatures:* □ *47.8° C. (118° F.);* △ *32.2° C. (90° F.);* ○ *23.9° C. (75° F.).*

large initial differences between the two types of biscuits, indicating that this measurement might prove valuable in detecting subtle initial differences in biscuits, such as, differences in components, or differences in processing, e.g., baking temperatures. The trends of the curves with storage time at the different temperatures indicated considerable variation in samples of biscuits. In spite of this variation, for both types of biscuits there appears to be a slight increase in fluorescing substances with storage time (1 to 5 photofluorometer units) and this increase appears to be most pronounced at the highest temperature. Although these results showed no great promise, this method was observed to be useful in subsequent studies on the keeping quality of biscuits (5).

* *Values kindly contributed by Mr. G. A. Grant of these laboratories.*

Discussion

Only a limited number of materials was investigated in the survey described here. However, this survey does indicate that fluorescence development is not peculiar to dried whole egg powder, nor is it confined to materials of high protein content. It is evident that fluorescence measurements are not likely to be a satisfactory quality test for dried milk powder or soya flour but may prove useful as a measure of quality for dehydrated pork, dried banana, dried parsnips, ration biscuits, and butter. This test may also detect reversion in hydrogenated linseed oil shortening.

References

1. Cook, W. H. and White, W. H.   Can. J. Research, D, 19 : 53-60.   1941.
2. Pearce, J. A.   Can. J. Research, C, 21 : 57-65.   1943.
3. Pearce, J. A.   Can. J. Research, D, 21 : 98-107.   1943.
4. Pearce, J. A.   Can. J. Research, D, 21 : 394-404.   1943.
5. Pearce, J. A. and Marshall, J. B.   In preparation.
6. Pearce, J. A. and Thistle, M. W.   Can. J. Research, D, 20 : 276-282.   1942.
7. Pearce, J. A., Thistle, M. W., and Reid, M.   Can. J. Research, D, 21 : 341-347.   1943.
8. Thistle, M. W., Pearce, J. A., and Gibbons, N. E.   Can. J. Research, D, 21 : 1-7.   1943.
9. Thistle, M. W., Reid, M., and Gibbons, N. E.   Can. J. Research, D, 21 : 267-269.   1943.
10. White, W. H.   Can. J. Research, D, 19 : 278-293.   1941.
11. Wright, N. C.   J. Dairy Research, 4 : 122-141.   1932.

DRIED WHOLE EGG POWDER

XIV. EFFECTS OF LOW TEMPERATURE, LOW MOISTURE CONTENT, CARBON DIOXIDE PACK, AND COPPER CONTAMINATION ON KEEPING QUALITY[1]

By M. W. Thistle[2], W. Harold White[3], Margaret Reid[2], and A. H. Woodcock[4]

Abstract

As shown by objective tests of quality, egg powder slowly deteriorated even at temperatures as low as −40° C. Low moisture content had a marked preservative action, but powders containing 1 4% volatile materials suffered some deterioration when held at 37° and 48° C. The use of a carbon dioxide pack afforded some measure of protection against heat deterioration, particularly on the solubility of the powder.

Copper contamination had no demonstrable effect on quality, as measured by potassium chloride and fluorescence values, on powders stored at 21° C. for three months, even in the presence of oxygen. The fat fraction showed no evidence of peroxide oxygen development.

Introduction

In the course of work previously reported (7, 10, 11) several points were suggested for further investigation. It was considered of interest to assess the effects of lower moisture content (10) and lower storage temperatures (11) than had been used previously, since these two factors appeared to be most important in lengthening storage life. A carbon dioxide pack showed promising preservative action (11) and merited more detailed examination. Finally even low grade egg powders showed marked resistance to oxidative changes (7): however, in view of the notable effect of copper in accelerating oxidative changes in dried milk (2) a study was indicated on the effect of copper contamination of egg powders. The present series of experiments was designed to extend previous information on all these points.

1. Quality Changes in Egg Powder Stored at Low Temperatures

Since egg powder deteriorated somewhat at 7.1° C. (45.0° F.) (11), information was sought on the quality changes, if any, occurring at still lower storage temperatures.

The egg powder used was from a commercial source and had an initial moisture content of 4.2%; moisture measurements (6) showed no increase during storage and handling. All operations previous to storage were carried

out at 7.1° C. Samples of powder were sealed in tin cans, and stored at
−40.0°, −17.8°, and 4.4° C. (−40.0°, 0.0°, and 40.0° F.) for periods of one,
two, four, and eight months. Quality changes were followed by means of
potassium chloride values (6), fluorescence measurements (3, 4, 5, 6), and
palatability ratings (4, 6).

Results

The results are shown in Table I. On the basis of the potassium chloride
values, no particular effect is evident at the end of four months' storage;
however, at the end of eight months, all samples had deteriorated, particularly
at the highest temperature   It is evident that some deterioration took place
even at −40.0° C.

TABLE I

QUALITY CHANGES IN EGG POWDER STORED AT LOW TEMPERATURES

Measurement	Storage temperature, °C.	Storage period, months					Replicate error
		Initial	1	2	4	8	
Potassium chloride value, %	−40.0	69.6	70.5	66.7	68.9	62.8	
	−17.8	68.0	70.0	69.6	70.0	61.7	1.2
	4.4	67.8	69.4	67.2	68.8	54.7	
Fluorescence, units	−40.0	15.0	17.0	17.8	17.9	20.6	
	−17.8	15.2	16.9	17.6	18.1	21.8	0.5
	4.4	15.0	16.6	18.4	19.0	24.5	
Palatability ratings (6-man panel)	−40.0	7.7	7.9	8.0	7.8	8.2	
	−17.8	7.6	7.9	8.0	—	8.3	0.7
	4.4	7.6	8.0	7.3	8.3	8.7	

These results were confirmed by the fluorescence values; moreover this
more sensitive test gave some evidence of deterioration even after one month's
storage at these low temperatures.

The palatability test, as used here, was not sensitive enough to detect these
minor differences in good quality powders. This is in agreement with previous
work (6).

· It is concluded that good quality egg powders deteriorate slowly even at
temperatures as low as −40.0° C.

2. Effect of Low Moisture Content on Keeping Quality

In an earlier communication (10) it was shown that lowering the moisture
and volatile content of egg powder improved its keeping quality, the lowest
moisture and volatile content used being 2%. The present experiment was
designed to test the efficacy of levels below 2%.

The moisture content of a sample of commercial egg powder was reduced
by methods already described (10) from an initial value of 4.4% to 1.4%.

Samples of powder at both normal and low moisture levels were stored in tin cans for 30 days at 37.2° C. (99.0° F.) and for 15 days at 47.8° C. (118.0° F.). Fluorescence measurements were made at three-day intervals during these storage periods.

Results

The results are presented in Fig. 1.   It can be seen that fluorescence development was much slower in powder at 1.4% moisture than in similar powder containing 4.4% moisture.   However, even at the low level of 1.4% moisture, egg powder suffers deterioration by heat in a relatively short time.   It may be inferred that reduction of the moisture level of commercial powders to the lowest practicable level will be useful in improving the keeping quality of dried eggs, but that this process will by no means render the product imperishable.
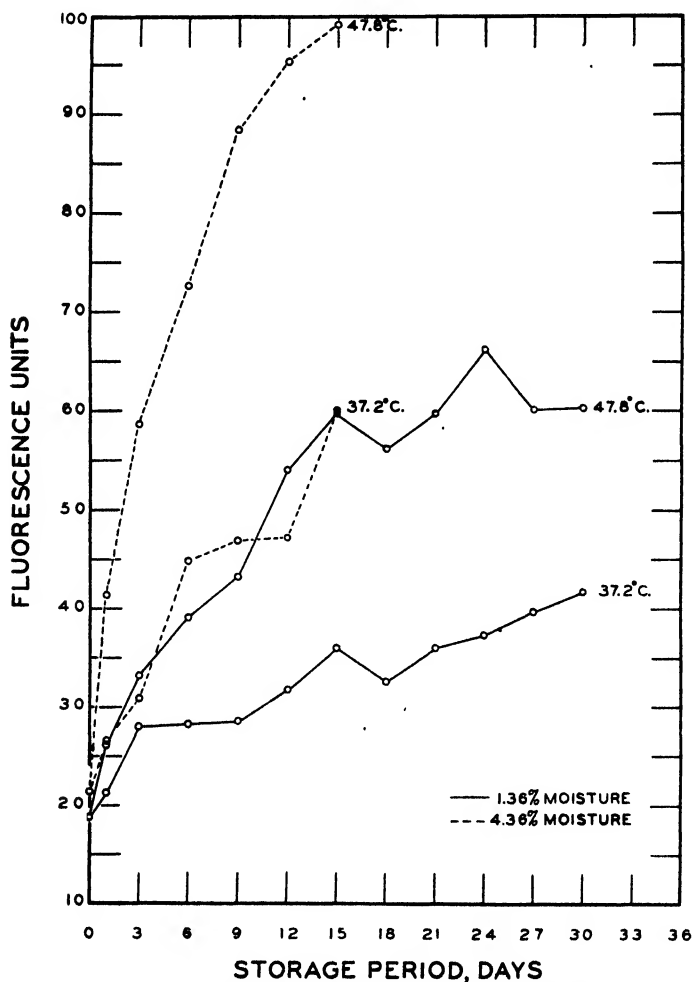
FIG. 1.   *Storage behaviour of egg powders with average and with low moisture contents.*

3. Effect of Carbon Dioxide on Keeping Quality

Earlier work indicated that carbon dioxide exerted a beneficial action on the keeping quality of dried eggs (11). The present experiment was designed to test this effect in greater detail.

Commercial dried egg powder of 3.7% moisture content was gas-packed with carbon dioxide in tin cans. Air and gas-packed samples were stored at 23.9° C. (75.0° F.) for 1, 2, 4, 8, 16, and 32 wk.; and at 47.8° C. (118.0° F.) for 1, 2, 4, 8, 16, 32, and 64 days. Quality was assessed by means of potassium chloride values, refractometric values (9), fluorescence values, and palatability ratings.. At the end of storage, analyses showed the following gas composition in the headspace:

Gas	24° C.	48° C.
Carbon dioxide	98.1%	97.6%
Oxygen	0.1%	0.2%
Nitrogen	1.8%	2.2%

Results

The results are presented in Fig. 2. The potassium chloride values indicate that the solubility of egg powder was retained better at both storage temperatures by packing in carbon dioxide. Confirmatory evidence is offered by the refractometric values and the greater cake volume (Fig. 3) exhibited by carbon-dioxide–packed samples.

In the powders held at 23.9° C. for eight months, fluorescence development in gas-packed samples was retarded. However, in the samples held under more rigorous conditions (47.8° C.), fluorescence development was retarded for the first few weeks of storage only, after which it was enhanced by the presence of carbon dioxide. The palatability of gas-packed samples was judged superior when the powder was held at 23.9° C., however at 47.8° C. the palatability ratings were higher for the first half of the storage period only, after which they fell to the same low level as that of the air-packed powders.

It was previously noted that solubility and flavour quality normally were correlated (6); apparently the association is disrupted by the use of carbon dioxide, suggesting that chemical changes take place in gas-packed powders at high temperatures.

It may be noted that packing egg powder in an atmosphere of carbon dioxide resulted in lengthening the storage life when ordinary temperatures were used. At higher storage temperatures the solubility of the powders was preserved, but the flavour quality was retained for a short period only.

It is concluded that the use of carbon dioxide in packaging dried egg powder retards heat deterioration, and in conjunction with other preservative measures may be useful in prolonging the storage life of dried egg.
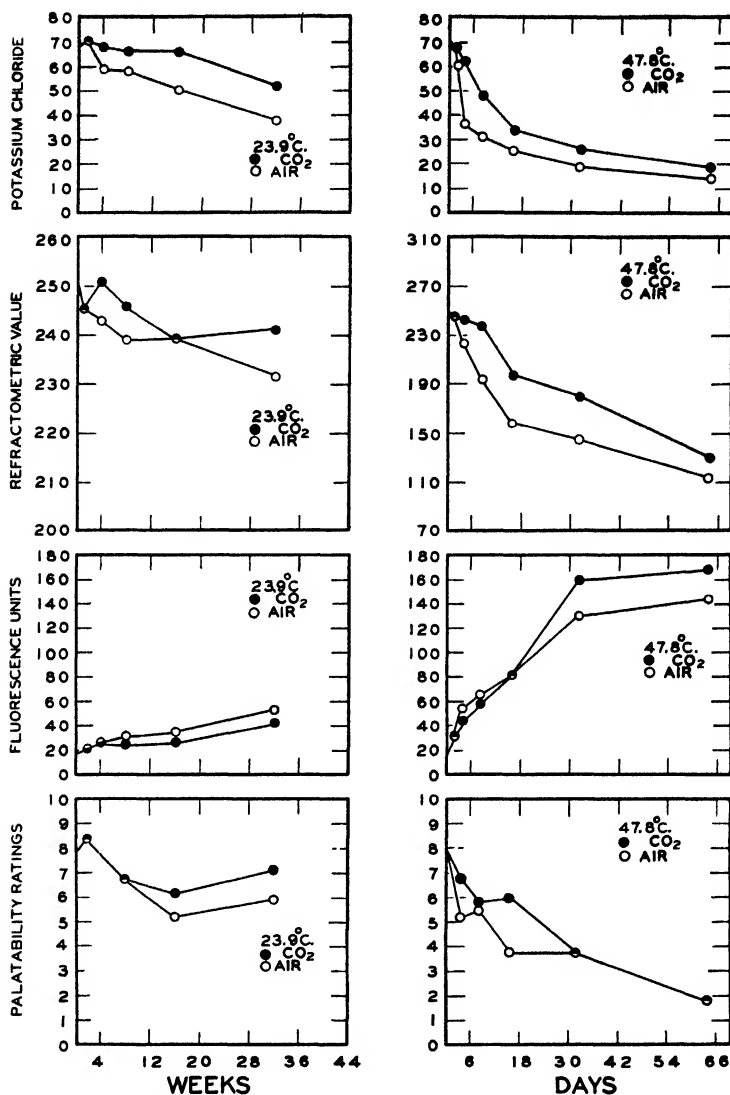
FIG. 2. *Storage behaviour of egg powders packed in air and in carbon dioxide.*

4. Effect of Small Amounts of Copper on the Stability of Vacuum-Dried Whole Egg Powder

Since all egg-drying plants are not provided with stainless steel equipment, some information was desirable on the influence of small amounts of copper in dried egg, such as might occur from contamination with copper or brass equipment.

Copper contamination was secured by pouring egg melange through a double thickness of new, fine-mesh copper screen, just prior to drying by the vacuum-ice method. Six batches of melange were treated by passing 0, 1,

PLATE I
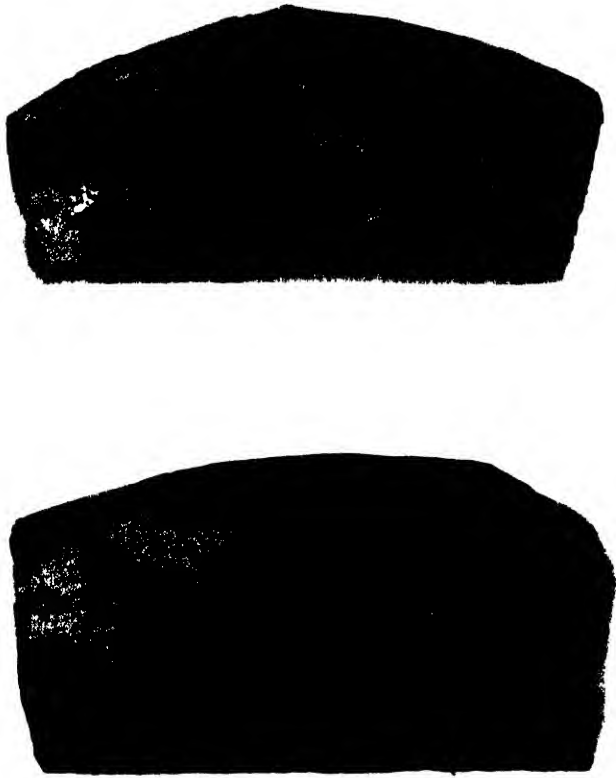
FIG. 3. Baking tests on egg powders held for eight months at 23 9° C. The upper loaf was made from carbon dioxide packed material and the lower from powders stored in air.

2, 4, 8, and 16 times through the copper screen. (One passage gave approximately $1\frac{1}{2}$ p.p.m. of added copper in the resulting dried egg.) Samples of dried egg from each batch were canned in both air and oxygen and stored at 21.1° C. (70.0° F.) for three months. At the end of storage, quality of the samples was assessed by means of potassium chloride and fluorescence values. Deterioration of the fat fraction was assessed by peroxide oxygen determinations (8).

The method used for determining copper consisted of wet oxidation (digestion with nitric, sulphuric, and perchloric acids) of dried egg samples to produce inorganic copper salts. The residue was treated by a tentative A.O.A.C. method (1) for determining copper in water analyses, except that visual colour comparisons were replaced by measurements made on the Evelyn photoelectric colorimeter.

Results

The results are given in Table II. Considering the differences between methods of packing, the mean potassium chloride values show that oxygen-packing accelerated deterioration. The difference between mean fluorescence values is small. The uniformly lower moisture content of the oxygen packed powder is due to repeated evacuation of the tins during gas-packing.

TABLE II

STABILITY OF EGG POWDER CONTAMINATED WITH COPPER AND STORED THREE MONTHS AT 21.1° C.

Sample No.*	Copper, p.p.m.	Packed in air			Packed in oxygen		
		Moisture, %	Potassium chloride value	Fluor-escence value	Moisture, %	Potassium chloride value	Fluor-escence value
0	1.8	3.68	69 1	20 8	2.96	57.8	23.0
1	3.3**	3.16	74.4	21.8	3.02	54.8	23.2
2	4.4	.5.12	65 2	25.4	4 74	49 4	25.6
4	7.8**	4.74	62.6	23.4	3.96	50.8	22.8
8	13.8	3.02	68.6	21.6	2.68	58.8	21.0
16	25.6	3.22	66.3	22.0	2.86	54.4	23.0
Mean	—	3 82	68 6	22.5	3.37	54 3	23.1

* *Number of passages of egg melange through a copper screen prior to drying and storage.*
** *Calculated values.*

NOTE—*The potassium chloride value and fluorescence methods were not available when this study was begun. However, the fluorescence of fresh vacuum-ice–dried egg powder is usually 12 units, and the potassium chloride value is usually about 80%.*

Differences due to the level of copper contamination were negligible. Oxygen appeared to be equally harmful in the presence and absence of copper; and the lowest potassium chloride values and the highest fluorescence values occur in Samples 2 and 4, rather than at the higher copper levels. Moreover, these effects can be accounted for by the higher moisture contents of Samples

2 and 4. The marked effect of moisture content on quality of egg powder has been demonstrated (10).

No evidence could be obtained of any peroxide oxygen formation. This is in agreement with previous work (4, 7).

It is concluded, therefore, that copper contamination under these conditions has little or no demonstrable effect on the stability of egg powder.

References

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis. 5th ed. A.O.A.C., Washington, D.C. 1940.
2. HOLLENDER, H. A. and TRACY, P. H. J. Dairy Sci. 25 : 249-274. 1942.
3. PEARCE, J. A. Can. J. Research, D, 21 : 98-107. 1943.
4. PEARCE, J. A. and THISTLE, M. W. Can. J. Research, D, 20 : 276-282. 1942.
5. PEARCE, J. A., THISTLE, M. W., and REID, M. Can. J. Research, D, 21 : 341-347. 1943.
6. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E. Can. J. Research, D, 21 : 1-7. 1943.
7. THISTLE, M. W., REID, M., and GIBBONS, N. E. Can. J. Research, D, 21 : 267-269. 1943.
8. WHITE, W. H. Can. J. Research, D, 19 : 278-293. 1941.
9. WHITE, W. H. and GRANT, G. A. Can. J. Research, D, 21 : 203-210. 1943.
10. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 211-222. 1943.
11. WHITE, W. H., THISTLE, M. W., and REID, M. Can. J. Research, D, 21 : 271-276. 1943.

SMOKED MEATS

II. DEVELOPMENT OF RANCIDITY IN SMOKED AND UNSMOKED WILTSHIRE BACON DURING STORAGE[1]

By W. Harold White[2]

Abstract

Spoilage in the fat of smoked and unsmoked Wiltshire backs and gammons, stored at temperatures of −18°, −9°, −1°, and 7° C. for approximately four months, was assessed by determinations of peroxide oxygen and free fatty acid contents. Smoked bacon can be stored satisfactorily for at least two months at −1° to − 18° C. whereas unsmoked bacon was usually rancid after one month. Spoilage due to the formation of excessive quantities of free fatty acids was found to be of little importance.

Introduction

There is normally little occasion for the storage of Canadian Wiltshire bacon in England since it is usually consumed within a relatively short period after its arrival. However, such a need may occur during the war period. Consequently, it is essential that adequate information be available on the most suitable storage conditions. While bacon is less susceptible to protein spoilage than pork, the curing salts normally employed in its manufacture have a pro-oxidant effect on the fat and tend to hasten the development of rancidity. It has been shown previously that both slime formation and fat spoilage can be materially retarded by smoking the bacon prior to storage (5). The investigation reported here was undertaken to determine the effect of storage temperature and of smoking on the quality of Wiltshire backs and gammons.

Peroxide oxygen and free fatty acid formation in the fat, and bacterial growth and the colour and colour stability of the lean meat, were used as criteria of quality. Data on the bacteriological and colour measurements will be reported in subsequent papers.

Material and Methods

The investigation was divided into two parts. In the first, the material consisted of the right and left sides of four hogs, selected for uniformity in size and quality. After cure the sides were allowed to drain and partially

---

mature for five days at 3.3° C. (38° F.), and the back and gammon were removed from each side. After allotting two backs at random to each of the storage temperatures of −18°, −9°, −1°, and 7° C. (0°, 15°, 30°, and 45° F.), each back was divided into two approximately equal portions, one of which was smoked at an air temperature of approximately 60° C. (140° F.) for 14 hr. This permitted a comparison to be made, with the same side, of the effect of smoking on changes during storage.

In order to minimize differences due to position, and the period to which any one cut surface was exposed during storage, each half back was subsequently divided into five equal portions, one of which, selected at random, was removed for study after the desired storage period. Prior to storage each of the five subsamples were pressed closely together, and the sample as a whole wrapped in sterile brown paper to minimize surface bacterial contamination. Desiccation during storage was minimized by storing the product in loosely closed metal containers.

The relative susceptibility to spoilage of the fat of backs and gammons was determined by smoking one gammon from each of the four hogs under the conditions described previously. Two gammons, one smoked and one unsmoked from the same hog, were held at each of the storage temperatures. This permitted a direct comparison to be made of the effect of smoking on material from the same hog. Prior to storage each gammon was divided into five subsamples and otherwise treated similarly to the backs.

The second part of the investigation was undertaken to determine if the size of the piece of bacon smoked had any effect on its subsequent storage behaviour. The relatively greater surface to volume ratio in small pieces might reasonably affect the rate of heat transfer, the extent of smoke deposition and the degree of desiccation of the meat. To permit this comparison the entire right and left sides of eight hogs were smoked under the conditions described previously, the backs removed, and each divided into six pieces for study during storage. Two smoked and the corresponding two unsmoked backs from the same hogs were held at each of the four storage temperatures. This permitted comparison of the effect of smoking on changes during storage in the fats of backs from the same hog.

Oxidative and hydrolytic changes in the fat were assessed from the peroxide oxygen and free fatty acid contents, respectively. Previous reference has been made to the methods employed (4). These determinations were made on the fat of both smoked and unsmoked half-backs and of gammons after 21, 42, 70, and 98 days for product stored at −1°, −9°, and −18° C., and after 11, 21, 32, and 42 days for product held at 7° C. In studying the effect of the size of the cut smoked, peroxide oxygen determinations alone were made on the back fat at the times mentioned above, and in addition after 130 days' storage at −1°, −9°, and −18° C. and 71 days at 7° C.

Results

Peroxide Oxygen Formation

Back Fat

The results obtained for peroxide oxygen formation in the fat of both small cuts and whole backs of smoked and unsmoked Wiltshire bacon during storage are given in Table I as average values for each treatment over all others studied. Because of the differences in sampling times it is to be noted that direct comparisons cannot be made between the data obtained for 7° C. and those for the lower temperatures, nor between small cuts and whole backs.

TABLE I

Mean values for the effect of storage period, temperature, and smoking on peroxide oxygen formation in the fat of Wiltshire back bacon

Temperature

°C.	7	−1	−9	−18
Mean peroxide oxygen content of:				
Small cuts[1, 2]	3.9	4.7	4.9	6.4
Whole backs [1, 2]	5.3	10.9	11.9	6.7

Smoking

Treatment	Smoked	Unsmoked
Mean peroxide oxygen content of:		
Small cuts[1]	2.8	7.8
Small cuts[2]	1.2	6.6
Whole backs[1]	5.9	13.7
Whole backs[2]	3.8	6.8

Storage period

Days	0	11	21	32	42	70	98	130
Mean peroxide oxygen content of:								
Small cuts[1]	0 88	—	1 9	—	5 2	7.1	11.5	—
Small cuts[2]	0.84	2.1	2.4	8.2	5.9	—	—	—
Whole backs[1]	0.84	—	0.96	—	7.7	12.3	17.5	19.7
Whole backs[2]	0.94	1.2	1.8	2.9	2 0	23.2	—	—

[1] *Mean, as ml. 0.002 N sodium thiosulphate over all other conditions studied for product stored at −1°, −9°, and −18° C.*

[2] *As for [1], but on data obtained at 7° C. only.*

While peroxide oxygen formation usually proceeded at a slower rate at the lower storage temperatures, the differences between the mean values at the various temperatures were small.

Smoking was very effective in retarding the development of rancidity in back fat. Examination of the detailed data shows that at all temperatures the unsmoked fat was usually slightly or definitely rancid after 21 to 42 days' storage, whereas that of most of the smoked bacon was in a satisfactory condition after 42 to 70 days. This behaviour is in marked contrast to that of pork for which it has been shown that a temperature of $-18°$ C. gives satisfactory protection for 48 weeks (2, 3).

The mean peroxide oxygen content increased progressively with the storage period. The results indicate that, on the average, the induction period of peroxide oxygen formation was between 21 and 42 days.

While the small cuts and the entire backs of smoked bacon behaved generally in a comparable manner, peroxide oxygen formation was greater in the fat of entire backs. However, the differences were not statistically significant. Possible factors contributing to this behaviour, such as differences in the extent of smoke deposition, etc., have been mentioned in the preceding discussion.

Details of the effect of storage temperature and time on peroxide oxygen formation in the fat of both smoked and unsmoked back bacon are illustrated in Fig. 1. In its preparation the data for the two experiments were averaged in order to present the most reliable information. From this it may be seen that, even when smoked and stored at $-18°$ C., incipient oxidative rancidity
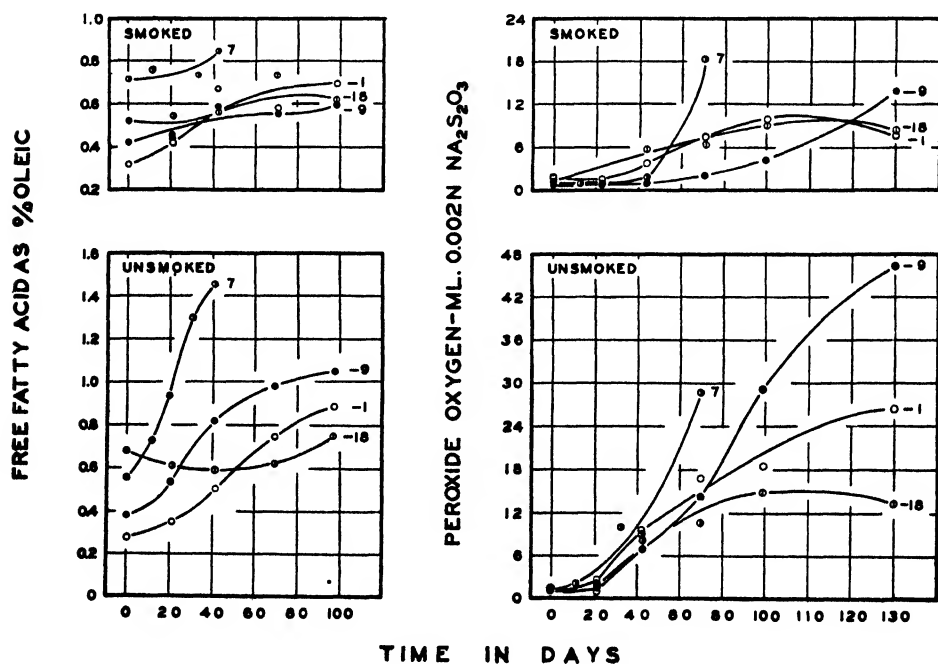
FIG. 1. *Peroxide oxygen and free fatty acid formation in the fat of smoked and unsmoked back bacon during storage at 7°, −1°, −9°, and −18° C.*

was evident in back bacon after a period of 40 to 70 days. The reasons for the variable behaviour with temperature are unknown.

The data for the peroxide oxygen content of the back fat could not be used directly for statistical analysis because of the variable manner in which different sides responded to the same treatment. This difficulty was overcome by transforming the data to the corresponding logarithms prior to analysis (1). The results of such analyses showed that smoking and the storage period were the most important factors studied for both small cuts and whole backs. The significant difference between hogs, observed for product stored at 7° C., is presumably due to inherent variations and not to experimental treatment since the material within each experiment was smoked at the same time and under the same conditions.

. TABLE II

ANALYSES OF VARIANCE FOR THE EFFECT OF STORAGE PERIOD, TEMPERATURE, AND SMOKING ON PEROXIDE OXYGEN FORMATION IN THE FAT OF WILTSHIRE BACK BACON

Source of variance	Small cuts		Whole backs	
	D.f.	Mean sq.	D.f.	Mean sq.
Material stored at −1°, −9°, and −18° C.				
Temperature	2	0.799	2	0.337
Between hogs within temperature	3	0.136	3	0.170
Smoking	1	3.80**	1	2 98**
Smoking × temperature	2	0 185	2	0.449*
Smoking × between hogs within temperature	3	0.032	3	0.015
Time	3	1.91**	5	8.26**
Time × temperature	6	0.215	10	0.195**
Time × smoking	3	0 517*	5	0.226**
Time × temperature × smoking	6	0.161	10	0.096*
Residual	18	0 153	30	0.034
Analytical error	48	0.002	72	0.005
Material stored at 7° C.				
Smoking	1	3.42**	1	0.860*
Between hogs	1	0 405*	1	0.533*
Time	3	0.195	5	2.06**
Time × smoking	3	0 548**	5	0.089
Residual	7	0.052	11	0.109
Analytical error	16	0 002	24	0.002

 * *Indicates 5% level of significance.*
 ** *Indicates 1% level of significance.*

Gammon Fat

The effect of the various factors studied on peroxide oxygen formation in the fat of gammons was similar to that described for the backs (Table III). The results of analyses of variance of the data are given in Table IV. It is

TABLE III

MEAN VALUES FOR THE EFFECT OF STORAGE PERIOD, TEMPERATURE, AND SMOKING ON PEROXIDE OXYGEN FORMATION IN THE FAT OF WILTSHIRE GAMMONS

Temperature

° C.	7	−1	−9	−18
Mean peroxide oxygen content[1, 2]	2.1	4.4	2.5	14.3

Smoking

Treatment	Smoked	Unsmoked
Mean peroxide oxygen content[1]	4.0	10.1
Mean peroxide oxygen content[2]	0.84	3.34

Storage period

Days	0	11	21	32	42	70	98
Mean peroxide oxygen content[1]	0 89	—	1.7	—	9.4	7.9	15.5
Mean peroxide oxygen content[2]	0.87	1.3	0.84	1.1	6.4	—	—

[1] *Mean, as ml. 0.002 N sodium thiosulphate, over all other conditions studied for product stored at −1°, −9°, and −18° C.*

[2] *As for [1], but on data obtained at 7° C. only.*

to be recalled that the smoked and unsmoked gammons studied at each temperature were from the same hog. Consequently, the observed differences with temperature also include variations between gammons from different hogs. Thus, inherent differences in the stability of the fat from different hogs may possibly account for the abnormally high values obtained at −18° C. Other than this, back and gammon fat behaved generally in a comparable manner during storage. Smoking caused a marked retardation in peroxide oxygen formation, and thus gives protection from spoilage to the fat of both backs and gammons.

FREE FATTY ACID FORMATION

Back Fat

The mean free fatty acid content of back fat was usually small (Table V), indicating that spoilage due to the formation of excessive quantities of free fatty acids during storage is of little importance. A similar behaviour was

TABLE IV

ANALYSES OF VARIANCE FOR THE EFFECT OF STORAGE PERIOD,
TEMPERATURE, AND SMOKING ON PEROXIDE OXYGEN FORMATION
IN THE FAT OF WILTSHIRE GAMMONS

Source of variance	D.f.	Mean sq.
Material stored at −1°, −9°, and −18° C.		
Temperature	2	809
Smoking	1	559
Smoking × temperature	2	57.9
Time	4	433*
Time × smoking	4	115*
Time × temperature	8	136*
Residual	8	26.8
Analytical error	30	0.397
Material stored at 7° C.		
Smoking	1	31.4
Time	4	22.9
Time × smoking	4	21.1
Analytical error	10	0.353

* *Indicates 5% level of significance.*

TABLE V

MEAN VALUES FOR THE EFFECT OF STORAGE PERIOD, TEMPERATURE, AND SMOKING ON FREE
FATTY ACID FORMATION IN THE FAT OF WILTSHIRE BACK BACON

Temperature

° C.	7	−1	−9	−18
Mean free fatty acid content[1, 2]	0.86	0.55	0.64	0.62

Smoking

Treatment	Smoked	Unsmoked
Mean free fatty acid content[1]	0.54	0.66
Mean free fatty acid content[2]	0.71	1.01

Storage period

Days	0	11	21	32	42	70	98
Mean free fatty acid content[1]	0.43	—	0.47	—	0.63	0.71	0.77
Mean free fatty acid content[2]	0.63	0.75	0.74	1.03	1.15	—	—

[1] *Mean, as % oleic acid, over all other conditions studied for product stored at −1°, −9°, and −18° C.*

[2] *As for [1], but on data obtained at 7° C. only.*

observed for pork fat (2, 3).   The mean acid content showed some tendency to increase with increase in storage temperature but the differences were small. Smoking resulted in a slightly lower mean value of free fatty acid.   This is presumably due to the retardation by smoking of the agencies responsible for both oxidation and hydrolysis of the fat.   Of the periods studied the increase in free fatty acid content was on the average relatively greatest between 21 and 42 days.   It should be recalled that the stage of rapid formation of peroxide oxygen also occurred within this period.   Further details on the effect of the factors studied on free fatty acid formation may be seen in Fig. 1.

The results of an analysis of variance (Table VI) showed that storage time only had a significant effect on the formation of free fatty acids at temperatures of $-1°$, $-9°$, and $-18°$ C.   However, at $7°$ C. differences in the free fatty acid content attributable to smoking, time in storage, and variations in sides reached the level of statistical significance.

TABLE VI

ANALYSES OF VARIANCE FOR THE EFFECT OF STORAGE PERIOD, TEMPERATURE, AND SMOKING ON FREE FATTY ACID FORMATION IN THE FAT OF WILTSHIRE BACK BACON

Source of variance	D.f.	Mean sq.
Material stored at $-1°$, $-9°$, and $-18°$ C.		
Temperature	2	0.085
Between hogs within temperature	3	0.256
Smoking	1	0.417
Smoking × temperature	2	0.101
Smoking × between hogs within temperature	3	0.117
Time	4	0.520*
Time × temperature	8	0.061
Time × smoking	4	0.056
Time × temperature × smoking	8	0.053
Residual	24	0.101
Analytical error	60	0.004
Material stored at 7° C.		
Smoking	1	0.859**
Between hogs	1	1.63**
Time	4	0.381*
Time × smoking	4	0.236
Residual	9	0.077
Analytical error	20	0.001

* Indicates 5% level of significance.
** Indicates 1% level of significance.

TABLE VII

MEAN VALUES FOR THE EFFECT OF STORAGE PERIOD, TEMPERATURE, AND SMOKING ON FREE
FATTY ACID FORMATION IN THE FAT OF WILTSHIRE GAMMONS

Temperature

° C.	7	−1	−9	−18
Mean free fatty acid content[1,2]	0.57	0.59	0.77	0.54

Smoking

Treatment	Smoked	Unsmoked
Mean free fatty acid content[1]	0.67	0.59
Mean free fatty acid content[2]	0.63	0.51

Storage period

Days	0	11	21	32	42	70	98
Mean free fatty acid content[1]	0.42	—	0.45	—	0.72	0.70	0.85
Mean free fatty acid content[2]	0.37	0.44	0.53	0.62	0.88	—	—

[1] *Mean, as % oleic acid, over all other conditions studied for product stored at −1°, −9°, and −18° C.*

[2] *As for* [1], *but on data obtained at 7° C. only.*

TABLE VIII

ANALYSES OF VARIANCE FOR THE EFFECT OF STORAGE PERIOD,
TEMPERATURE, AND SMOKING ON FREE FATTY ACID
FORMATION IN THE FAT OF WILTSHIRE GAMMONS

Source of variance	D.f.	Mean sq.
Material stored at −1°, −9°, and −18° C.		
Temperature	2	0.296
Smoking	1	0.117
Smoking × temperature	2	0.046
Time	4	0.406*
Time × smoking	4	0.007
Time × temperature	8	0.106**
Residual	8	0.016
Analytical error	30	0.001
Material stored at 7° C.		
Smoking	1	0.069
Time	4	0.157
Time × smoking	4	0 026
Analytical error	10	0.002

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

Gammon Fat

The mean free fatty acid content of the fat of the gammons was, like that of the backs, low and somewhat variable (Table VII). Variations in storage temperature had little effect. In contrast to the backs, the mean free fatty acid content of smoked gammon fat was greater than that of the unsmoked. The reason for this is not apparent. The greatest increase with time was again observed to occur between 21 and 42 days' storage. An analysis of variance indicated that the only statistically significant variables were storage period and the differential effect of temperature at different storage times (Table VIII).

Acknowledgment

References

1. COCHRAN, W. G.   Empire J. Exptl. Agr. 6 : 157-175.   1938.
2. COOK, W. H. and WHITE, W. H.   Can. J. Research, D, 19 : 53-60.   1941.
3. WHITE, W. H.   Can. J. Research, D, 19 : 96-103.   1941.
4. WHITE, W. H.   Can. J. Research, D, 19 : 278-293.   1941.
5. WHITE, W. H., GIBBONS, N. E., WOODCOCK, A. H., and COOK, W. H.   Can. J. Research, D, 20 : 263-275.   1942.

SMOKED MEATS

III. EFFECT OF MATURATION PERIOD ON QUALITY[1]

By W. Harold White[2], A. H. Woodcock[3], and N. E. Gibbons[4]

Abstract

Wiltshire-cured sides were matured at −1.1° C. (30° F.) for periods of 1 to 25.5 days prior to smoking at 60° C. (140° F.). The effect of the length of the maturation period on quality was assessed by flavour tests; and by determination of surface bacterial growth, peroxide oxygen formation in the fat, and changes in colour and colour stability of the lean meat during storage at −1.1° C. subsequent to smoking.

By all criteria of quality used, differences attributable to variations in the length of the maturation period were usually small and showed no consistent trend. However, there was some indication that a maturation period of about 10 to 15 days was most suitable.

Introduction

The results of extensive investigations on the flavour of Canadian Wiltshire bacon (9, 10) showed that the English consumer would prefer a product containing less salt than that supplied prior to the war. Subsequent studies indicated that a milder cured bacon, when smoked, would be in a satisfactory condition after shipment to England (8), and that smoking materially retarded the development of rancidity in bacon fat (7). With such information available on the desirability of smoking bacon prepared for export purposes, it was believed important to determine the most suitable time after cure for doing this, i.e. the length of the maturation period. The present paper describes an investigation to determine the effect of maturation period on flavour quality of Wiltshire backs and gammons and on surface bacterial growth, colour changes, and development of rancidity in the fat during storage after smoking.

The period of maturation is believed to improve the quality of the product, especially with respect to colour uniformity and stability, and the development of the typical bacon flavour. Little is known concerning the actual nature of the changes occurring. However, these presumably include alterations in the protein constituents and greater uniformity in the distribution of the curing salts. Moreover, it is generally considered that such changes are either retarded or prevented after bacon is smoked.

Materials and Procedure

The right and left sides of 16 hogs of uniform size and good quality were pumped and cured for nine days according to regular commercial practice. After cure, the sides were drained at about 4.4° C. (40° F.) for one day, wiped, divided into groups of four at random, baled, and stored at −1.1° C. (30° F.). One bale was removed from storage after each of 1, 4.5, 8, 11.5, 15, 18.5, 22, and 25.5 days from cure. The backs and gammons were removed from the sides and without washing or soaking were smoked at an air temperature of 60° C. (140° F.) for approximately 18 hr.

Bacteriological Measurements

The effect of smoking on surface bacterial content was determined by making counts before and after smoking on the rib surfaces of the sides matured for 1, 11.5, 18.5, and 25.5 days from cure.

To obtain information on the effect of maturation period on subsequent keeping quality a portion approximately 6 in. in length was taken from the front end of each back after smoking, wrapped in sterile, brown paper and stored at −1.1° C. in loosely covered containers. Surface bacterial counts were made on these samples after 29, 55, 97, and 139 days from cure. The procedures followed for securing and preparing the meat samples for bacteriological examination have been described (4).

Measurement on Fat

The peroxide oxygen content of the fat of the small cuts of bacon mentioned above was determined (6) at the same intervals during storage as the surface bacterial counts.

Colour Measurements

Colour measurements were made on portions approximately 6 in. in length taken from the rear of each back after smoking, after 25.5 days from cure, and finally after 30 days' storage at −1.1° C. from time of smoking. The determinations were made with a photoelectric colour comparator described previously (11, 12). Moreover, an estimate of colour stability was obtained by exposing the sample, after the initial measurement, to the atmosphere for 70 hr. at 7.1° C. (45° F.) and remeasuring the colour.

Flavour Tests

Flavour tests were made on both backs and gammons. The ribs and all bones were removed from the backs, and the shank bone from the gammons. Slices of the back (0.15 in. thick) and of the gammon (about 0.38 in. thick) were removed from the front or rear positions at random. The samples of back and gammon were prepared for tasting by grilling at 232° C. (450° F.) for six and eight minutes, respectively. When not required the gammons and backs were doubly wrapped in waxed paper and overwrapped with brown, and stored at −1.1° C.

The flavour tests were made progressively throughout the experiment in such a manner that the group of sides representing any particular maturation

period were compared, after smoking, with all groups matured for shorter periods. For example, in the first set of tests the 1- and 4.5-day groups were compared; in the second set, the 1-, 4.5-, and 8-day groups, etc., thus giving seven different sets in all. In addition, the final set of comparisons, which included all maturation periods, was repeated after storage of the backs for 17 and 36 days at $-1.1°$ C.

An outline of the design of the various sets of flavour tests is given in Table I. Further details may be obtained from Fisher (3). In each set of tests the selection of the backs and gammons for the maturation periods compared was at random, except that each side within any one period was represented at least once. Tests on the backs and gammons were made on successive mornings. A member of the panel who tasted any particular combination of backs also tasted the same combination of gammons.

TABLE I

DESIGN OF FLAVOUR TESTS ON WILTSHIRE BACON MATURED FOR VARIOUS PERIODS AT $-1.1°$ C.

Maturation periods[1] compared	Design of experiment	Number of tasters	Number of different combinations of maturation periods	Number of samples compared in any one combination
1, 4.5	Complete block	6	1	2
1, 4.5, 8	Complete block	6	1	3
1, 4.5, 8, 11.5	Duplicated, incomplete block	8	4	3
1, 4.5, 8, 11.5, 15	Single, incomplete block	10	10	3
1, 4.5, 8, 11.5, 15, 18.5	Single, incomplete block	10	10	3
1, 4.5, 8, 11.5, 15, 18.5, 22	Duplicated, incomplete block	14	7	3
1, 4.5, 8, 11.5, 15, 18.5, 22, 25.5	Single, incomplete block	14	14	4

[1] *Time in days from removal of bacon from cure to smoking.*

The members of the panel, consisting of male employees of these laboratories, were required merely to place the samples in order of preference, giving, where possible, reasons for the preference. The tasters were permitted to function in their own manner, and were free to state that no distinction was possible between any two or more samples. Cold water was provided for each taster and most took advantage of it.

Numerical values were assigned to the preference ratings so that the intervals between successive scores were such as to transfer the ordinal numbers obtained in scoring to normal deviates amenable to analysis of variance (3). Thus, for comparisons between two samples these were $+0.56$ and -0.56; for three samples $+0.85$, 0, and -0.85; and for four samples, 1.03, 0.30, -0.30, and -1.03. If any two or more samples were indistinguishable, each was assigned the average of the scores corresponding to the positions occupied by these samples in the order of preference.

Results

Bacterial Growth

Surface bacterial growth between the time of wiping and smoking of the sides was small and, within the limits studied, was independent of the length of this period (Table II). Smoking markedly reduced the surface counts, in agreement with a previous observation (8). However, the length of the maturation period had no apparent systematic effect on the extent of bacterial reduction when the sides were smoked.

TABLE II

EFFECT OF SMOKING ON SURFACE BACTERIAL COUNTS OF BACON MATURED
FOR VARIOUS PERIODS AT $-1.1°$ C.

Maturation period, days	Log_{10} of number of organisms per sq. cm.[1]		
	After wiping	Before smoking	After smoking
1	4.11	4.11	1.43
11.5	4.70	4.81	0.90
18.5	4.60	4.77	0.93
25.5	4.42	4.50	2.19

Analysis of variance[2]

Source of variance	D.f.	Mean square
Maturation period	3	0.552
Error	12	0.421
Smoking	1	81.186**
Smoking $\times$ maturation period	3	1.304**
Error	12	0.194

[1] *Mean values for four sides.*
[2] *On data obtained before and after smoking only.*
** *Exceeds 1% level of statistical significance.*

Mean values and an analysis of variance for the effect of the duration of the maturation period on surface bacterial growth during storage at $-1.1°$ C. subsequent to smoking are given in Table III. Surface counts, as averaged over all maturation periods, increased at a relatively uniform rate during

TABLE III

EFFECT OF PERIOD OF MATURATION ON SURFACE BACTERIAL GROWTH ON
SMOKED BACON DURING STORAGE AT $-1.1°$ C.

Maturation period, days	Days from cure				Mean[2]
	29	55	97	139	
	Log_{10} of number of organisms per sq. cm. after storage[1]				
1	1.91	3.75	5.20	4.36	3.81
4.5	2.39	3.57	5.15	6.07	4.30
8	2.71	2.46	4.20	6.42	3.95
11.5	1.12	1.48	2.41	4.79	2.45
15	1.43	0.75	4.11	4.30	2.52
18.5	1.90	3.53	6.34	6.89	4.66
22	1.91	1.14	2.47	5.01	2.63
25.5	2.10	2.40	3.62	5.73	3.46
Mean[3]	1.94	2.39	4.19	5.39	—

Analysis of variance

Source of variance	D.f.	Mean square
Maturation period	7	11.618*
Error	24	3.610
Storage period	3	82.306**
Storage period × maturation period	21	2.296
Error	72	1.742

[1] *Mean values for four sides.*
[2] *Necessary difference, 5% level of statistical significance: 0.93.*
[3] *Necessary difference, 5% level of statistical significance: 0 66.*
* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% level of statistical significance.*

storage, with differences in count between 55, 97, and 139 days reaching the level of statistical significance. Least growth occurred during storage on sides matured for 11.5, 15, and 22 days. While this behaviour may be a direct reflection of the maturation period only, the lack of systematic differences between the various periods suggests that it is more probably due to such causes as unknown variations in the smoking procedure, bacterial flora, or the initial, general condition of the sides.

Visual examination of the samples after the various storage periods indicated the presence of a slight amount of slime on four samples after 55 days, and a slight to heavy growth on five samples after 139 days. Some moulds were present at 97 and 139 days. There appeared to be no relation between maturation period and extent of slime and mould growth.

Fat Stability

The peroxide content of the fat of the samples of back bacon increased significantly during storage, with the greatest change occurring between 97 and 139 days (Table IV). The length of the maturation period was without

TABLE IV

EFFECT OF PERIOD OF MATURATION ON PEROXIDE OXYGEN FORMATION IN FAT
OF SMOKED BACON DURING STORAGE AT $-1.1°$ C.

Maturation period, days	Days from cure				Mean
	29	55	97	139	
	Peroxide oxygen content, ml. 0.002 N Na$_2$S$_2$O$_3$				
1	0.86	0.85	1.77	4.01	1.88
4.5	0.95	2.92	3.36	5.69	3.23
8	0.85	2.45	2.87	7.82	3.49
11.5	1.02	1.38	3.72	7.20	3.33
15	0.98	3.62	3.89	7.68	4.04
18.5	0.89	1.28	1.85	5.34	2.34
22	0.90	1.01	2.26	6.94	2.78
25.5	0.92	3.11	2.90	7.93	3.72
Mean	0.92	2.08	2.83	6.57	—

Analysis of variance

Source of variance	D.f.	Mean square
Maturation period	7	8.38
Error	24	4.86
Storage period	3	191.37**
Storage period $\times$ maturation period	21	2.30
Error	72	2.91

** Exceeds 1% level of statistical significance.

significant effect. It would appear that Wiltshire bacon can be held at $-1.1°$ C. for periods as long as 25.5 days prior to smoking without harm to the keeping quality of fat during subsequent storage.

Colour and Colour Stability

Brightness, i.e. the total light scattered, of the freshly cut meat surface after smoking bore no significant relation to the length of the maturation period (Tables V and VI), but on the average decreased significantly during subsequent storage at $-1.1°$ C. The maturation period had significant differential effect on brightness changes occurring during storage. The values for samples matured for 3.5 and 8 days were significantly the lowest after smoking and increased slightly on storage, whereas those for the other periods decreased. Brightness after smoking was greatest for the 25.5-day group but decreased the most during storage. It would appear that samples matured for 1 and 15 days had on the average the most satisfactory brightness.

Data on the relative amount of light scattered in the various wave bands studied are given also in Tables V and VI. Variations in the colour intensities with maturation period were somewhat irregular. However, in general, violet intensity was least for sides matured from 8 to 15 days and greatest for

TABLE V

EFFECT OF PERIOD OF MATURATION ON COLOUR CHANGES OF SMOKED BACON STORED AT −1.1° C.

Factor	Mean brightness[1]	Mean scatter[1]								
		3850–4340 Å	4340–4580 Å	4580–4870 Å	4870–5250 Å	5250–5560 Å	5560–5840 Å	5840–6140 Å	6140–6440 Å	Above 6440 Å
Maturation period, days										
1	28.7	45.7	120.6	124.3	175.8	112.5	176.8	103.3	69.7	71.6
4.5	27.2	46.2	123.5	122.9	174.1	112.5	172.7	103.9	70.8	73.9
8	23.7	44.9	123.0	121.7	173.8	111.3	171.8	104.2	73.1	77.1
11.5	26.1	39.4	121.3	122.7	175.6	114.7	176.5	105.3	70.4	74.3
15	28.5	43.6	121.5	123.8	174.1	115.0	178.3	102.9	69.9	70.8
18.5	26.6	47.1	120.3	123.4	176.2	111.8	176.2	103.1	70.3	71.7
22	27.9	49.5	120.5	123.3	174.4	111.7	176.2	102.5	69.7	72.3
25.5	29.4	47.9	123.9	123.1	174.9	113.4	176.3	101.2	68.8	70.4
Necessary difference[2]	—	4.4	—	—	—	—	3.9	—	—	—
Storage time										
After smoking	28.5	47.2	124.9	122.9	174.9	113.0	172.6	102.5	69.9	72.5
25.5 days after cure	26.6	46.7	120.2	122.1	173.8	111.8	175.1	104.6	72.0	73.7
30 days after smoking	26.7	42.8	120.4	124.4	175.9	113.8	179.1	102.8	69.1	72.1
Necessary difference[2]	0.9	2.7	1.3	1.5	1.5	1.2	2.4	1.1	1.5	—

[1] *Mean values for all other conditions over the whole experiment.*
[2] *Necessary difference required to exceed 5% level of statistical significance.*

TABLE VI

ANALYSES OF VARIANCE FOR THE EFFECT OF MATURATION PERIOD ON THE COLOUR CHANGES OF SMOKED BACON STORED AT −1.1° C.

Source of variance	D.f.	Total brightness	Mean square								
			3850–4340 Å	4340–4580 Å	4580–4870 Å	4870–5250 Å	5250–5560 Å	5560–5840 Å	5840–6140 Å	6140–6440 Å	Above 6440 Å
Maturation period	7	39.07	112.91**	25.43	13.00	9.57	22.57	58.57*	17.71	18.86	58.86
Error	24	33.98	30.37	36.29	11.58	22.08	15.58	31.88	22.21	35.21	70.58
Storage time	2	38.08**	185.14**	231.50**	45.50**	35.00*	34.00**	340.50**	41.00**	73.00**	24.00
Storage time × maturation period	14	27.63**	120.34**	12.86	15.50*	20.29*	25.36**	23.93	5.50	7.79	11.64
Error	48	3.29	29.16	6.94	6.94	9.42	5.54	19.13	5.13	8.35	10.81

* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% level of statistical significance.*

22 days. Although differences in the blue region were small, sides matured for 22 and 25.5 days usually had higher intensity values. There was some indication that maturation periods of 4.5 and 8 days were associated with minimum green and maximum red intensities. During storage at −1.1° C. for 30 days after smoking, colour intensities on the average decreased in the

violet and blue, increased in the green, and showed little change in the red. Analyses of variance showed that the observed differences reached the level of statistical significance for maturation period in one band in each of the violet and green regions and for storage in all bands except that above 6440 Å. These primary factors also showed significant differential effects.

The results of the measurements on colour stability are given in Tables VII and VIII. Differences due to the length of the maturation period again

TABLE VII

EFFECT OF PERIOD OF MATURATION ON THE MEAN VALUES OF THE
COLOUR STABILITY OF SMOKED BACON STORED AT $-1.1°$ C.

Factor	Mean change in brightness[1]	Mean change in scatter[1]								
		3850–4340 Å	4340–4580 Å	4580–4870 Å	4870–5250 Å	5250–5560 Å	5560–5840 Å	5840–6140 Å	6140–6440 Å	Above 6440 Å
Maturation period, days										
1	−0.40	−0.17	−5.17	−0.17	+4.33	+6.17	+12.42	−5.25	−6.50	−5.00
4.5	+0.13	+0.92	−7.00	+0.33	+2.75	+2.92	+10.25	−3.33	−5.17	−3.50
8	−0.31	−0.67	−3.83	+3.67	+4.00	+2.67	+6.92	−2.08	−5.08	−5.58
11.5	−0.15	+3.17	−5.83	+1.08	+3.67	+2.67	+9.25	−4.25	−4.83	−3.92
15	−0.56	+2.75	−3.92	−0.42	+4.50	+1.08	+7.58	−2.08	−5.67	−3.17
18.5	−0.38	−1.67	−3.92	+2.58	+3.08	+4.33	+9.75	−3.08	−6.83	−4.58
22	−0.67	+0.33	−2.42	+1.33	+5.42	+3.58	+7.58	−3.75	−6.17	−6.17
25.5	−0.33	−0.50	−4.48	+1.50	+3.00	+1.42	+5.83	−1.41	−3.33	−2.42
Necessary difference[2]	—	—	—	—	—	—	3.40	2.10	—	0.70
Storage time										
After smoking	−0.58	−1.44	−4.94	+1.44	+4.81	+4.13	+11.13	−3.91	−6.44	−5.13
25.5 days after cure	−0.20	+0.09	−2.50	+1.59	+3.06	+2.72	+7.03	−3.50	−5.84	−3.53
30 days after smoking	−0.22	+2.91	−6.25	+0.41	+3.66	+2.47	+7.93	−2.06	−4.06	−4.22
Necessary difference[2]	—	—	2.00	—	—	—	2.10	1.30	1.40	—

[1] *Mean values for all other conditions over the whole experiment.*
[2] *Necessary difference required to exceed 5% level of statistical significance.*

TABLE VIII

ANALYSES OF VARIANCE FOR THE EFFECT OF MATURATION PERIOD ON
THE COLOUR STABILITY OF SMOKED BACON STORED AT $-1.1°$ C.

Source of variance	D.f.	Mean square									
		Total brightness	3850–4340 Å	4340–4580 Å	4580–4870 Å	4870–5250 Å	5250–5560 Å	5560–5840 Å	5840–6140 Å	6140–6440 Å	Above 6440 Å
Maturation period	7	0.72	0.58	23.85	22.87	9.71	31.71	54.00**	19.43**	14.83	19.40*
Error	24	0.59	51.98	16.64	8.36	7.38	15.08	18.92	5.50	9.47	7.36
Storage time	2	1.53	38.14	115.88**	18.20	25.50	25.50	148.00**	30.00*	48.89**	20.40
Storage time × maturation period	14	0.49	214.09**	24.32	13.27	19.71*	19.79*	47.64**	13.21*	19.92**	11.87
Error	48	0.52	47.14	15.40	16.59	10.19	8.06	15.94	7.13	7.46	7.96

* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% level of statistical significance.*

TABLE IX

EFFECT OF PERIOD OF MATURATION ON THE FLAVOUR OF SMOKED WILTSHIRE BACKS AND GAMMONS

Material	Periods compared, days	Total flavour score[1] Days							
		1	4.5	8	11.5	15	18.5	22	25.5
Backs	1 and 4.5	+0.56 (1)	−0.56 (2)	—	—	—	—	—	—
	1 to 8	+0.85 (1)	+0.43 (2)	−1.28 (3)	—	—	—	—	—
	1 to 11.5	−2.13 (3)	−4.25 (4)	+1.28 (3)	+5.10 (1)	—	—	—	—
	1 to 15	+0.43 (2)	−1.70 (4)	0.00 (1)	+3.83 (1)	−2.55 (5)	—	—	—
	1 to 18.5	0.00 (4)	−1.28 (5)	+2.98 (1)	+1.28 (2)	−3.83 (6)	+0.85 (3)	—	—
	1 to 22	−2.13 (5)	+0.85 (3)	−0.43 (4)	−5.10 (6)	+4.25 (1)	+2.98 (2)	−0.43 (4)	—
	1 to 25.5— Trial I	−2.79 (7)	−0.90 (5)	−2.75 (6)	+5.39 (1)	+0.04 (4)	+3.35 (2)	+1.07 (3)	−3.41 (8)
	Trial II	−0.04 (4)	−6.26 (8)	−2.10 (6)	−0.28 (5)	−4.12 (7)	+4.59 (1)	+3.77 (3)	+4.44 (2)
	Trial III	+0.62 (3)	−4.68 (8)	−1.10 (5)	+0.43 (4)	−2.15 (6)	+6.87 (1)	−2.25 (7)	+2.25 (2)
Gammons	1 and 4.5	+2.24 (1)	−2.24 (2)	—	—	—	—	—	—
	1 to 8	+1.70 (1)	−1.70 (3)	0.00 (2)	—	—	—	—	—
	1 to 11.5	−0.85 (3)	−2.55 (4)	+3.83 (1)	−0.43 (2)	—	—	—	—
	1 to 15	+0.85 (2)	−2.13 (5)	−0.43 (4)	0.00 (3)	+1.70 (1)	—	—	—
	1 to 18.5	+2.13 (3)	0.00 (3)	−2.98 (5)	−0.85 (4)	+2.98 (1)	0.00 (3)	—	—
	1 to 22		−0.43 (4)	+2.55 (2)	−2.55 (5)	+6.38 (1)	−3.40 (6)	−4.68 (7)	—
	1 to 25.5— Trial I	+5.71 (1)	+4.27 (2)	+2.04 (4)	+0.50 (6)	+3.39 (3)	+0.97 (5)	−4.63 (7)	−12.23 (8)
	Trial II	+2.88 (1)	−0.02 (5)	−0.45 (6)	−2.90 (7)	+0.90 (4)	+2.30 (2)	−4.40 (8)	+1.70 (3)
	Trial III	+3.63 (2)	−0.07 (4)	−2.66 (7)	+1.40 (3)	−0.64 (5)	+3.78 (1)	−3.78 (8)	−1.65 (6)

[1] Figures in parentheses indicate the order of preference within any one set of comparisons.

showed no definite trend. Brightness stability appeared to be best for the 4.5-day period and poorest for 22 days, but the differences were not significant. There was some indication that sides matured from 1 to 11.5 days had on the average greater negative values in the blue and greater positive values in green than those held for longer periods prior to smoking. A similar comparison for changes in red intensity shows that the values are negative and of approximately the same average magnitude.

During storage at $-1.1°$ C. for 30 days after smoking, the stability values, as averaged over all maturation periods became less negative or more positive in brightness and the violet and red bands; and more negative or less positive in the blue and green bands. Certain of these changes were statistically significant.

Flavour

Mean values and analyses of variance for the flavour scores of both backs and gammons are given in Tables IX and X. No distinct relation was evident between the length of the maturation period and flavour quality of either the

TABLE X

ANALYSES OF VARIANCE FOR THE EFFECT OF PERIOD OF MATURATION ON THE
FLAVOUR OF SMOKED WILTSHIRE BACKS AND GAMMONS

Periods compared, days	Source of variance	D.f.	Mean square	
			Backs	Gammons
1 and 4.5	Maturation period	1	0.052	0.836
	Error	11	0.508	0.494
1 to 8	Maturation period	2	0.105	0.241
	Error	22	0.499	0.701
1 to 11.5	Maturation period	3	1.569	0.689
	Error	29.	0.548	0.689
1 to 15	Maturation period	4	0.605	0.208
	Error	36	0.605	0.710
1 to 18.5	Maturation period	5	0.610	0.426
	Error	35	0.491	0.517
1 to 22	Maturation period	6	1.045	1.638*
	Error	50	0.583	0.540
1 to 25.5, Trial I	Maturation period	7	0.823	2.841**
	Error	77	0.646	0.527
1 to 25.5, Trial II	Maturation period	7	1.376*	0.537
	Error	77	0.631	0.697
1 to 25.5, Trial III	Maturation period	7	1.018	0.641
	Error	77	0.638	0.722

* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% level of statistical signifiacnce.*

TABLE XI

MEAN VALUES AND ANALYSES OF VARIANCE FOR THE CONTENTS OF CURING SALTS AND OF
MOISTURE IN SMOKED WILTSHIRE BACKS AND GAMMONS
MATURED FOR VARIOUS PERIODS

Maturation period, days	Sodium chloride[1], %		Sodium nitrate[1], %		Sodium nitrite[1], p.p.m.		Moisture[1], %	
	Back	Gammon	Back	Gammon	Back	Gammon	Back	Gammon
1	6.54	5.15	0.20	0.28	576	179	64.6	70.0
4.5	6.04	5.43	0.24	0.28	175	125	60.4	67.2
8	7.67	5.47	0.15	0.36	42	108	61.5	68.2
11.5	6.60	4.66	0.30	0.22	21	99	60.0	70.2
15	5.87	5.66	0.19	0.33	10	98	59.5	68.5
18.5	6.39	4.92	0.17	0.33	47	45	64.8	69.5
22	7.08	5.67	0.32	0.30	5	24	61.2	69.5
25.5	7.54	5.91	0.25	0.35	14	43	62.2	68.7

Analyses of variance

Constituent	Source of variance	D.f.	Mean square	
			Backs	Gammons
Sodium chloride	Maturation period	7	0.889	0.351
	Error	8	0.645	0.465
Sodium nitrate	Maturation period	7	0.007	0.004
	Error	8	0.017	0.008
Sodium nitrite[2]	Maturation period	7	0.831*	0.161
	Error	8	0.204	0.100
Moisture	Maturation period	7	8.068	2.095
	Error	8	6.651	3.053

[1] *Mean values for two sides.*

[2] *Values transformed to logarithms prior to statistical analysis.*

* *Exceeds 5% level of statistical significance.*

backs or gammons. However, there was some indication that the flavour of the backs was preferable after they were matured for 11.5 or 18.5 days, and that of the gammons, after 1 or 15 days. Thus, it would appear that the most suitable period for the development of the desired flavour in whole Wiltshire sides is about 15 days. However, it should be noted that the observed differences for the most part were not statistically significant.

To determine whether observed differences in palatability were due to variations in the content of curing salts, the concentration of sodium chloride, nitrate, nitrite, and of moisture were determined at the conclusion of the flavour tests for two backs and two gammons selected at random from those available for each maturation period. The methods employed have been described previously (5). The sodium chloride content (Table XI) was

somewhat higher and that of moisture lower than had been observed previously for Canadian Wiltshire bacon (12). Unavoidable desiccation during storage may have been partially responsible for this. Differences in the moisture and salt content between sides matured for various periods were not statistically significant. This suggests that any preferences indicated in flavour quality were due to other causes than the concentration of the above constituents.

Acknowledgments

References

1. COOK, W. H. and CHADDERTON, A. E. Can. J. Research, D, 18 : 149-158. 1940.
2. COOK, W. H. and WHITE, W. H. Can. J. Research, D, 18 : 135-148. 1940.
3. FISHER, R. A. and YATES, F. Statistical tables for biological, agricultural and medical research. Oliver and Boyd, Ltd., Edinburgh and London. 1938.
4. GIBBONS, N. E. Can. J. Research, D, 18 : 202-210. 1940.
5. WHITE, W. H. Can. J. Research, D, 17 : 125-136. 1939.
6. WHITE, W. H. Can. J. Research, D, 19 : 278-293. 1941.
7. WHITE, W. H. Can. J. Research, F, 22 : 97-106. 1944.
8. WHITE, W. H., GIBBONS, N. E., WOODCOCK, A. H., and COOK, W. H. Can. J. Research, D, 20 : 263-275. 1942.
9. WHITE, W. H., WINKLER, C. A., and COOK, W. H. Can. J. Research, D, 19 : 213-224. 1941.
10. WINKLER, C. A. and COOK, W. H. Can. J. Research, D, 19 : 157-176. 1941.
11. WINKLER, C. A., COOK, W. H., and ROOKE, E. A. Can. J. Research, D, 18 : 435-441. 1940.
12 WOODCOCK, A. H. Can. J. Research, D, 21 : 90-97. 1943.

AN AIR CONDITIONED TUNNEL FOR PROCESSING FISH

AND

THE DEHYDRATION OF FISH

BY

E. P. SIDAWAY AND O. C. YOUNG

CHANGES IN MOISTURE, MICRO-ORGANISMS AND VOLATILE BASES IN DEHYDRATED FISH DURING PROCESSING AND STORAGE

BY

H. L. A. TARR

AN AIR-CONDITIONED TUNNEL FOR PROCESSING FISH

As pointed out in a note on page 20 in the previous issue of these Progress Reports, the Pacific Fisheries Experimental Station has under way experiments concerned with the dehydration of some Pacific coast fishes, for which special equipment was being built. Keeping in mind general utility, the apparatus presented in pictorial projection in the accompanying diagram was decided upon. The only new units in the entire equipment are the rectangular tunnel, the conveyor system and the smoke producer. The other appliances, comprising the cooler, heater, fan, reduction gearing, controlling recorders, motors and piping were parts already on hand from previous smoking equipment installed in the Station's former quarters in Prince Rupert.

The accompanying diagram is self-explanatory, and it will be noticed that essentially the apparatus comprises a conveyor within a tunnel in which the air conditions can be controlled within the limits of the capacities of the various appliances involved. Nothing unique is claimed for the equipment, indeed if dehydration were the only objective, quite a different apparatus would have been evolved. Taking into account, however, the various uses to which the apparatus would be put, the illustrated equipment was chosen as being the most desirable. It will be recognized that the conveyor could have been placed in a vertical or sloping tunnel just as effectively as in the horizontal one shown, if floor space were at a premium. Furthermore in smoking establishments employing the kiln or chimney type of smoker, the conveyor system could be placed in the existing chimney.

The use of a conveyor system of some type was felt to be desirable to produce uniformity in the product whether it be a smoked or a dehydrated one. The speed of the conveyor is relatively unimportant. But it is important, however, to have every portion of the product go through identical conditions to induce uniformity. This has been achieved in the illustrated equipment as shown by results from both dehydrating and smoking experiments.

Since smoking is only a partial dehydration process, relatively lower temperatures are used than in dehydration processes. Consequently apparatus that is designed for both smoking and dehydrating must necessarily be more flexible than if designed for smoking only, particularly with regard to the heating appliances. Since the illustrated equipment is composed of old units taken from former smoking equipment, the heating capacity is somewhat low for some of the dehydration experiments planned; therefore auxiliary steam coils are being added for extension of these experiments. Furthermore as the tunnel was constructed for larger sprocket wheels than were subsequently supplied for the conveyor, a certain amount of the original space in the tunnel served no purpose, and is therefore being separated from the useful space by a core which directs the conditioned air past the materials on the conveyor and thereby increases the velocity of this air in the reduced, effective space in the tunnel.

The smoke producer is entirely different from the type employed at Prince Rupert and was designed by one of us (E.P.S.) as a result of experience gained elsewhere with fish smoking The producer consists of a large cylinder or combustion chamber of heavy black iron, in which the shavings

4

AIR CONDITIONED TUNNEL

SMOKE PRODUCER — 3'0" Diam

BAFFLED AIR COOLER

5 KW HEATER

Thermostat

Recirculation Duct

Loading Door

Dampers

Valves

Brine In / Out Brine

Note: All pipes 6 inches diameter. Damper in pipe to cooler not visible

2 HP Motor & Reducer Gear

CONVEYOR DUCT

Double Decked Carrier For Screen Trays

Cars

Recirculation Duct and Damper not visible

Damper

Discharge to Chimney

Fan — Driven by 3 HP Motor
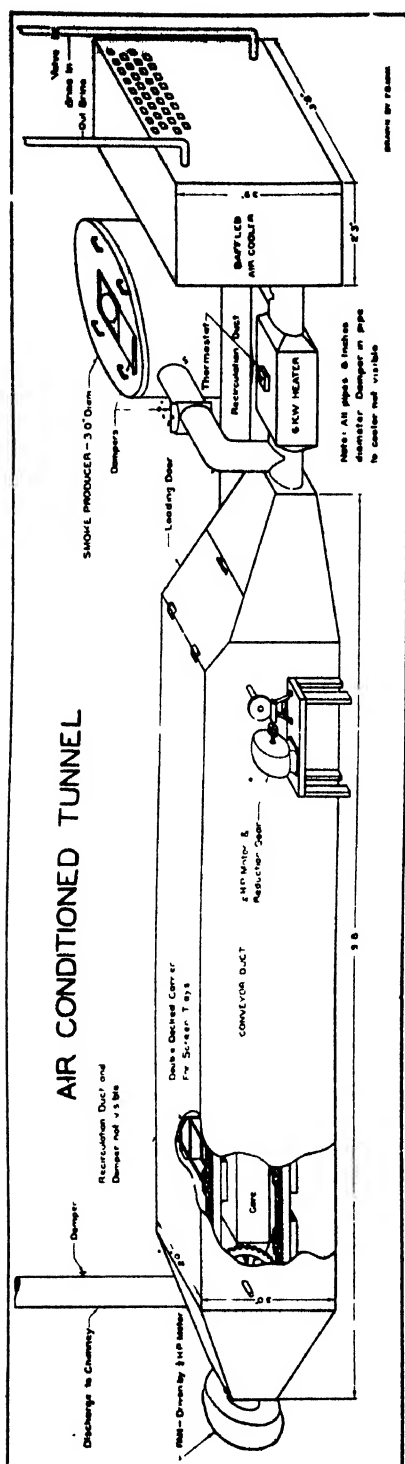
and sawdust fuel is placed. This fuel is packed to any desired depth around the sides, but is confined to a depth of several inches in the centre. The lid of this cylinder is tight fitting and has a centrally attached vertical pipe open at both ends and extending to within a few inches of the surface of the layer of shavings and sawdust with which the burner is charged. A slide damper over the upper end of this pipe controls the downward draft of air supplied to the burning materials in the combustion chamber. The products of combustion are drawn off through a horizontal pipe leading off near the top of the combustion chamber, and the rate at which these products are drawn off may be governed by a damper in this pipe.

Time has not permitted exhaustive tests with this piece of the equipment but the few tests already made indicate that the rate and degree of combustion can be closely controlled although some practice with the dampers is necessary to obtain the desired results.

The experiments thus far in both smoking and dehydrating have not entailed the use of the cooler, mainly because of the relatively high temperatures employed. So far the smoking experiments have actually been to test the equipment for the production of uniformity of colour in the smoked product and have been conducted at temperatures between 90° and 100° F. The dehydration experiments have all been conducted at a temperature of 120° F. and in all cases the relative humidity has been so low that the cooler was not needed. Both the cooling and the humidifying appliances (latter not yet installed) will be necessary for experiments where the careful control of humidity is essential.

As already intimated, we have nothing to report on smoking, other than that the equipment has come up to all expectations in the smoking tests made. However, since considerable data relating to the dehydration of the various kinds of fish have been obtained, these are being presented in an accompanying report in this issue. •

E. P. Sidaway
O. C. Young

THE DEHYDRATION OF FISH

For centuries the drying of fish has been practised all over the world, and statistics would probably show that the greatest bulk of fish is still preserved in this way. However, the traditional method of exposure to wind and sun is relatively slow and results in much loss through spoilage, lack of uniformity of the final product and the development of characteristic "cured" flavours not favoured by modern industrial populations, who have become accustomed to varieties of fresh, frozen or canned fish. Furthermore, drying yields a product that does not re-absorb its initial content of water nor does it revert to its original delicate glutinous texture.

The possibility of producing by a controlled process an improved dried fish acceptable to the British people has been under investigation for some time by the Department of Scientific and Industrial Research of Great Britain at their Torry Research Station. Here, C. L. Cutting and G. A. Reay obtained such promising results from some of their experiments that further investigations were encouraged, the whole matter being considered very important as a war measure.

At the outset, the British investigators felt that the drying of the raw fish would afford the best possible product, and to achieve this, drying was done under a high vacuum at temperatures below the freezing point. The results, however, were not at all satisfactory as the product did not reconstitute well on soaking, and the flesh was bleached, spongy and not very palatable. As chemical tests revealed that considerable irreversible alteration (denaturation) of the flesh proteins had taken place, the possibility of simpler methods of drying at higher temperatures and at atmospheric pressure suggested themselves.

Various methods of preparation were tried and the fish were dried by roller driers and by warm moving air. The most satisfactory results were obtained by mincing the raw fish, cooking for half an hour in steam at a pressure of from 2 to 3 lb. gauge, regrinding after cooking and then drying in moving air initially heated to 185° but having its temperature gradually lowered to 158° F. Under these conditions drying was accomplished in 4 hours and a product obtained that reconstituted well and subsequently received excellent reports from catering authorities in the Army, Navy and Air Forces in which samples were tested.

These results were of great importance to countries which export large quantities of salted, frozen and canned fish, particularly those countries subject to great losses in transport. Fisheries research organizations in the United States, Newfoundland and Canada at once set up pilot plants for the dehydration of the various kinds of indigenous fishes, and all are endeavouring to obtain solutions to their specific problems. Duplication of effort is avoided as far as possible by correspondence and by personal discussions such as took place during the recent visit of investigators from the Seattle branch of the United States Fish and Wildlife Service laboratories to this Station. But fisheries technologists realize the danger of generalization, therefore each species of fish is investigated separately.

The equipment used for dehydrating purposes in this Station, described in a separate report in this issue, is different from that used in the Seattle laboratory and also different from that used in the Newfoundland Fishery

7

Research Laboratory at St. John*.*. The main difference is that in the latter two laboratories the fish are stationary while at this Station a conveyor is used to keep the fish in motion.

As a result of various trials, in our experiments the following procedure was found to give the most satisfactory results generally:

(1) The dressed clean fish were steamed from 22 to 30 minutes at atmospheric pressure (212° F.).

(2) The fish were quickly skinned and deboned after their temperature had dropped sufficiently to make handling comfortable.

(3) The still warm flesh was pressed at approximately 100 lb. per sq. in., to remove expressible oil and water.

(4) The pressed cakes of flesh were ground in a meat grinder.

(5) The ground material was spread on wire screen trays at a loading of approximately 1 lb. per sq. ft. of screen area.

(6) Dehydration was effected in approximately 4 hours starting with a temperature of 120° F. and a relative humidity of 19%.

During cooking and any pressing of the cooked flesh of oily fish (*e.g.* herring) a considerable amount of oil and aqueous liquid is removed. The oil content of fish flesh constitutes an appreciable fraction of the energy-giving value of the flesh as a food, but some of this oil must be removed in preparation for dehydration in order to assure satisfactory keeping qualities for the dehydrated product. The oil expressed is, however, recoverable for other purposes. The loss of aqueous liquid during cooking and/or pressing results in that much less water having to be removed during the dehydrating, but at the same time incurs a slight loss of dissolved protein and other nitrogenous food materials. The food values in this liquid are also recoverable in the form of other products if economically warranted.

A breakdown of the various operations carried out, with the resultant losses, together with the total reduction in weight, the moisture remaining in the final product and the specific gravity of the product, is given in the accompanying table. It will be noticed that the total reduction in weight varies from 86.3% for herring to 94% for red snapper. The reduction in volume might be somewhat less than the weight reduction, depending entirely upon the form of the fresh fish from which the basis of comparison is drawn and the degree to which the dried product is compressed. Employing the results obtained for herring, one ton of round fish would produce approximately 274 lb. of dehydrated fish which would occupy about 5-1/3 cu. ft. of space if pressed at 500 lb. per sq. in. If this were packed in 1-lb. tall cans is would require approximately 336 cans or 7 cases of 48 cans each, whereas from 20 to 25 cases of 1 lb. tall cans would be required to contain the product resulting from the commercial canning of a ton of round herring. Taking the mean of the latter container requirements the saving in space due to dehydrating would be about 69%. This saving could be considerable increased, however, if the pressed dehydrated fish were packaged in rectangular cartons which lend themselves to more compact stowage. According to chemical analysis the protein content of the 7 cases of dehydrated herring would be greater than that of the 22½ cases of ordinary canned herring, for determinations made in our laboratories showed that the edible portion of the raw herring contained 16.4% protein while the dehydrated product from the same parcel of fish gave a value of 75.2% protein. Assuming no loss of protein in the canning process and assuming further that each can contained 1 lb. of edible fish, then the 22½ cases or 1080 lb. of ordinary canned herring would contain 177 lb. of protein, whereas the 7 cases or 274 lb. of dehydrated herring

Operation:	Herring (round)	Brill (round)	Grey cod (dressed)	Ling cod (dressed)	Red snapper (round)	Halibut (dressed)	Salmon (coho, round)	Dogfish (eviscerated only)
	%	%	%	%	%	%	%	%
Dressing	27.3	*	*	*	*	*	*	*
Filleting	*	56.8	*	42.7	72.1	40.5	47.3	*
Cooking	18.0	12.5	} 65.6	22.2	5.9	14.9	2.6	} 57.7
Deboning	5.2	*		*	*	*	*	
Pressing	15.2	8.5	7.1	*	*	*	*	14.2
Drying	20.6	15.0	19.9	25.1	16.0	33.7	36.7	15.9
Total reduction in weight	86.3%	92.8%	92.6%	90.0%	94.0%	89.1%	86.6%	87.8%
Moisture remaining in dehydrated fish	5%	5.5%	7%	8%	5%	†	5%	†
Specific gravity of dehydrated fish after pressing at 500 lb/sq. in.	0.82	0.73	0.71	0.71	0.6"	0.71	0.69	0.94

* Signifies operation not necessary for experimental product desired
† Signifies analysis not yet available.

would contain 206 lb. of protein, that is, over 16% more than the canned herring would contain. The greater *weight yield* of protein in the dehydrating process arises from the circumstances that less trimming and other losses of flesh are incurred in preparing the ton of round fish for dehydrating than for canning.

Comparisons similar to the above are being drawn up for the various types of frozen fish ordinarily exported but the studies have not advanced sufficiently to be included in this report. Investigations into the reconstitution of the final products, the storage at different temperatures and in different types of containers are being conducted also, but only simple cooking tests have been made.

Experiments so far show that the reconstitution or restoration of the dehydrated products to a form in which they may be used in the kitchen is best effected by the use of *cold* water. The products take up water readily, and in doing so swell greatly and regain the colour and texture they possessed before being dehydrated. In a number of experiments the dehydrated products were shown to reconstitute during 30 minutes soaking in cold water quite adequately, so that on the addition of a little wheat flour to act as a binder, the mixture when moulded into cakes or balls and fried with a little fat provided a very attractive and quite palatable dish. In the preparation of such dishes, it is to be borne in mind that the dehydrated product is already precooked.

The writers wish to thank Miss B. H. Morton of the staff of this Station for the protein determinations referred to in the above report.

Pacific Fisheries Experimental Station   O. C. Young
                                                  E. P. Sidaway

CHANGES IN MOISTURE, MICRO-ORGANISMS AND VOLATILE BASES IN DEHYDRATED FISH DURING PROCESSING AND STORAGE

Recently considerable work has been carried out in Great Britain on the production and storage of precooked dehydrated meat   The problem of storage and transport of this type of product is rather a new one for it is only recently that need for such information has arisen   So far as the writer is aware published information on the storage of dehydrated meat has as yet been limited to studies on the moisture content of the product when stored at different relative humidities and temperatures, with particular reference to the conditions favouring growth of moulds   However, it is anticipated that further studies will be made in order to ascertain whether or not alterations in such constituents of the meat as the proteins or fats occur during prolonged storage under different conditions   In recognition of these facts, and in view of the experiments which have been carried out in Great Britain, and recently at this Station, on methods of producing satisfactory precooked dehydrated fish, a study of the various microbiological, chemical and physical alterations which occur during production and storage of such a product has been commenced

The fish used in the experiments to be outlined was, unless otherwise stated, dehydrated according to the general procedure which was described in a recent article in these Progress Reports (No 56 pages 7-9, September, 1943)   It has been assumed that minor differences in dehydration procedure such as reasonable variations in the temperature and time used would be unlikely to affect significantly the results of the experiments described   The following method of expressing the results obtained has been used

Relative Humidity (R H)--Amount of water vapour present in the air expressed as a percentage of the amount of water vapour in that air if it were completely saturated with water vapour at the temperature stated

Numbers of bacteria or yeasts and moulds- Number of living (viable) organisms per ounce of fish

Volatile bases   Parts per million (p p m) parts by weight of fish   Ammonia (total volatile base minus trimethylamine) and trimethylamine have been determined   The method used was only sensitive to about 5 p p m in the case of ammonia and 20 p p m in the case of trimethylamine

Moisture –Percent of water present

Alterations in the Micro-organism and volatile Base Content of Fish During Processing.

Samples of fish were taken at different stages in the dehydration process and were analysed for bacteria and moulds and yeasts   The results, given in table I, show that while the flesh of freshly steamed fish has few or no living bacteria present, it becomes significantly contaminated during processing, especially during pressing and mincing   The apparent increase in micro-organisms during dehydration of the precooked minced flesh may in fact be due partly to growth and partly to a concentration as a result of shrinkage in weight and volume

16

Type of fish studied	Number of bacteria after:			Numbers of yeasts and moulds after:		
	cooking	pressing and mincing	dehydration	cooking	pressing and mincing	dehydration
Herring	400	20,000	480,000	—	—	—
Lingcod	—	2,700	26,000	—	230	630
Chum salmon	--	23,000	130,000	—	230	1,700
" "	0	11,000	51,000	0	230	1,300
" "	—	—	230,000	—	—	11,000*

* A large number of yeasts.

Analysis of one sample of chum salmon for volatile base content during processing gave the following results:

	Ammonia (p.p.m.)	Trimethylamine (p.p.m.)
Cooked minced fish	270	About 30
Dehydrated fish	460	Less than 20

Moisture, Bacterial and Volatile Base Content of Dehydrated Fish Stored at Different Relative Humidities

All the experiments were carried out at 77° F., for at this temperature the types of bacteria, yeasts and moulds associated with fish tend to multiply rapidly under appropriate conditions. The samples of dehydrated fish used were freshly prepared. Portions were placed in sterilized uncovered glass dishes in sealed glass vessels containing solutions of either calcium chloride or sulphuric acid of a concentration calculated to maintain the desired relative humidity at the temperature of the experiments. The results of certain experiments follow.

Moisture, Moulds and Bacteria and Volatile Bases in Dehydrated Coho Salmon, Lingcod and Chum Salmon Stored at Relative Humidities Between 100 and 65%

Experiments carried out in Great Britain, which have already been referred to briefly, showed that unless dehydrated meat (containing 40% fat) is stored at relative humidities *below* 75% (at temperatures between 68° and 77° F.), moulds are certain to develop. Similar experiments were made by the writer with dehydrated coho salmon and lingcod. The lingcod was dried for 24 hours at 115° F. in still air, had a very poor external appearance, and a high bacterial content due to these conditions favouring bacterial growth. Samples were stored as described above, the desired relative humidity being maintained with calcium chloride solutions. The moisture content and numbers of bacteria present were determined as soon as a given sample showed visible development of moulds, while those which showed no visible growth of moulds after 45 days were then examined. The results are given in table II. They indicate that moulds are unlikely to develop on dehydrated fish unless the relative humidity at which it is stored is 80% or over, corresponding to an average moisture content of about 17.5%, and even at this humidity mould growth would occur only slowly and to a limited extent.

17

Variety of dehydrated fish	R.H. at which sample was stored (%)	No. of days before visible mould appeared	Moisture (%)	No. of bacteria in sample (M=millions)
Coho salmon*	—	—	4.8	370,000
Lingcod*	—	—	2.6	450 M
Coho salmon	100	7	29.4**	12,000 M
Lingcod	100	7	28.3**	10,000 M
Coho salmon	90	9	27.6	5,100 M
Lingcod	90	9	30.0	7,100 M
Coho salmon	85	18	19.2	240,000
Lingcod	85	18	19.7	42 M
Coho salmon	80	34†	17.2	3,400
Lingcod	80	None in 45 days	17.9	6.7 M
Coho salmon	75	" "	13.5	12,000
Lingcod	75	" "	14.1	1.1 M
Coho salmon	70	" "	12.8	4,100
Lingcod	70	" "	13.1	5.4 M
Coho salmon	65	" "	11.8	1,500
Lingcod	65	" "	12.2	3.3 M

* Controls; determinations made on fish directly after dehydrating.
** In 12 days these samples, which were then extremely mouldy, contained 37.5% and 37.3% moisture, respectively.
† Only one small patch of mould developed.

In this connection it is of interest to note that British work already referred to showed that the critical moisture content at which moulds are just able to develop on dehydrated meat (containing about 40% fat) was 17% when calculated on a fat-free basis. It will be observed that a marked increase in bacterial numbers only occurred at R.H. values between 100% and 90%. At a R.H. of 85% there was a slight increase in the bacterial content of chum salmon, while in the case of the lingcod there was a decrease in the very high initial bacterial content. Evidently this humidity, which corresponds to a moisture content of about 19.5%, represents about the critical limit for development of bacteria on dehydrated fish. At R.H. values of 80% or lower the initial bacterial content decreased considerably.

An experiment similar to the above was made using dehydrated chum salmon, but in this case the volatile base content of the samples was determined as well. As far as the relations between R.H., moisture content and development of mould are concerned, the results (table III) were very similar to those of the foregoing experiment. Trimethylamine developed in all samples irrespective of whether or not bacteria or moulds increased. As yet no satisfactory explanation can be offered for this increase. There was also a slight increase in the ammonia content of some of the samples, and this was in many instances also apparently independent of the development of moulds or bacteria. It will be noticed that at R.H. values between 100% and 80% both increases and decreases in the ammonia content were found. In this region observed decreases may have been due to the utilization of ammonia by the moulds, and increases to bacterial action or to some other factor.

R.H. at which sample was stored (%)	No. of days before visible mould appeared	Moisture (%)	No. of bacteria (M=millions)	Amount of volatile bases in sample:	
				Trimethylamine	Ammonia
—	—	4.0*	650,000*	less than 20*	460*
100	6	33.1	1,900 M	220	750
90	8	23.2	45 M	80	85
85	17	19.0	30,000	180	218
80	35†	16.8	170,000	380	450
75	None in 35 days	13.6	42,000	360	550
70	"	12.1	33,000	500	790
65	"	11.0	150,000	500	660

* Control; determinations made on fish directly after dehydrating.
† Only a few patches of mould.

Moisture, Bacteria and Volatile Bases in Dehydrated Lingcod Stored at Relative Humidities Between 80% and 10%

Samples of dehydrated lingcod were stored over sulphuric acid solutions at relative humidities between 80% and 10%, and the numbers of bacteria, moisture content and volatile bases were determined after 30 days. The results, given in table IV, show that there is a steady increase in moisture content in the samples stored at R.H. values between 10% and 60%, but that at the higher humidities the rate of moisture uptake increases much more markedly. Similar results were obtained in the work with dehydrated meat previously cited. After storage for 30 days there was no important increase or decrease in the bacterial content; the observed changes were very probably due to chance sampling errors which are apparently rather high with material of this nature. There appeared to be a slight increase in the trimethylamine content, but not in the ammonia content. These samples, as well as those in the previous experiment, are being subjected to more prolonged storage in order to ascertain whether the changes noticed are thereby accentuated.

TABLE VI. Moisture content, numbers of bacteria and amount of volatile bases in dehydrated lingcod stored at relative humidities between 80% and 10%.

R.H. at which sample was stored (%)	Moisture (%)	No. of bacteria in sample	Amount of volatile bases in sample:	
			Trimethylamine	Ammonia
—	1.7*	130,000*	180*	280*
10	3.0	210,000	93	260
20	4.4	140,000	180	240
30	5.7	180,000	350	250
40	6.8	290,000	350	240
50	8.3	1,900,000	550	180
60	9.3	110,000	200	280
70	13.6	130,000	200	88
80	17.9	25,000	130	96

* Control; initial determinations made on freshly dehydrated fish.

19

Flavour Reversion in Linseed Shortening[1]

J. G. ARMSTRONG and W. D. McFARLANE
Department of Chemistry, Macdonald College (McGill University)
Ste. Anne de Bellevue, Quebec, Canada

Introduction

THIS study was undertaken in an effort to improve hydrogenated linseed oil for use as an edible fat to help meet the shortage in Canadian supplies. Preliminary work had indicated that although a shortening of apparently good quality could be produced, the products baked with this shortening, e.g., pastry or toasted bread, had a disagreeable flavour, commonly referred to as a reverted flavour.

The term "reverted" has been commonly used to describe a group of "off" odours which develop on heating or storing certain oils. Although it suggests the return of a flavour or odour which has previously been removed, this implication is incorrect and misleading, especially in the case of linseed shortening because the flavour and odour are different from any which were present either in the crude or the refined oil or in the freshly hydrogenated shortening. It should also be pointed out that, while reversion in an oil proceeds through a succession of different odours described by such terms as "grassy," "painty," etc., the organoleptic sensations of taste and odour associated with reverted linseed shortening seem to be unique and characteristic.

The problem of flavour reversion is not encountered with hydrogenated sunflower and cotton seed oils, so it would appear that reversion in linseed shortening must be associated either with linolenic acid which is present in linseed oil to about 40% and absent from the other oils, or with a constituent of the nonsaponifiable matter of linseed oil which is not present in the other oils. Lemon (4) first suggested that linolenic acid was the responsible constituent and has obtained spectrographic evidence to support his theory that in the hydrogenation of linseed oil the linolenic acid is saturated first at the $\triangle^{12,13}$ double bond to form an isomer of the common linoleic acid which, when heated, gives rise to the odoriferous product.

In this investigation a study has been made of the cause of flavour reversion and the possibility of producing a more acceptible shortening by modifying the customary steps in processing and by special treatments including the use of antioxidants.

Methods

REFINING

ALKALI, bisulphite and adsorbent-refining have been studied. In alkali refining, crude linseed oil was stirred rapidly at room temperature for 45 minutes with a slight excess of a 30% aqueous solution of sodium hydroxide, then stirred slowly at 65-70°C. for 30 minutes; allowed to settle overnight, and the soaps separated by filtration. In one experiment, samples of shortening were refined by carrying out the whole operation at 65°C. Samples of linseed oil were also refined with bisulphite in the same manner as in alkali refining but using 5 ml. of a 10% aqueous solution of

sodium bisulphite per 100 g. of oil instead of sodium hydroxide and with less vigorous agitation to avoid emulsions. The oil was separated by decantation and filtration.

Filtrol-carbon and a silica-gel-alumina preparation developed in this laboratory, were employed as adsorbents. The silica-gel-alumina adsorbent was prepared by grinding 100 g. of silica gel[2] to pass a 100-mesh sieve and adding, with stirring, a solution containing 55 g. aluminium nitrate nonahydrate in 500 ml. of absolute ethyl alcohol. The mixture was stirred in a large evaporating dish on a steam bath to remove the alcohol, then dried in an oven at 100°C. and finally heated in a muffle furnace at 750°C. for three hours. Refining with this adsorbent was carried out by allowing about 140 g. of oil to percolate through a one-inch layer of the adsorbent in a 2½-inch Buchner funnel. The Filtrol-carbon adsorbent was a mixture of a special Filtrol and the activated carbon, Darco G-60, in the proportions of 5:1. It was added to the oil at a level of 3.6%; the mixture agitated vigorously for 30 minutes at 65-70°C. and the oil separated by filtration.

HYDROGENATION AND DEODORIZATION

The catalyst was prepared by the method of Hilditch (3) employing the dry reduction of nickel carbonate but with silica gel (100 mesh) instead of kieselguhr for the base, and reducing at about 500°C instead of at 400-450°C. The catalyst was stored in a small amount of hydrogenated linseed oil or Blended Shortening. It contained about 28% nickel before mixing with the fat and about 14% after mixing.

An eccentric-driven shaker-type apparatus manufactured by the Standard Calorimeter Co., East Moline, Ill., was used for hydrogenation. The reaction took place in a pyrex glass bottle of 500-ml. capacity heated by a coil of nichrome wire wound around the outside of the bottle between layers of asbestos paper and the reaction temperature was adjusted by a light-bank rheostat. A charge of 100 or 200 g. of oil was placed in the reaction bottle with the catalyst (0.5-0.6% nickel for refined oils and 1.0-1.2% for crude oils) and hydrogenated at 180°C. with a hydrogen pressure of about two atmospheres. Electrolytic hydrogen was admitted from a small storage tank and after calibrating the apparatus the course of the reaction could be followed by noting the fall in hydrogen pressure. The extent of hydrogenation was always checked by determining the iodine number of the product.

Immediately after hydrogenation, all samples were deodorized by passing a jet of steam into the shortening for three hours at 200°C. and about 5 mm. pressure. The vacuum was then released by admitting nitrogen through the steam jet, and the shortening was clarified by filtering through silica gel adsorbent or Super-Cel Standard.

ANALYTICAL PROCEDURES

Acid values were determined by the A. O. A. C. method (8) but using 5 g. samples of the oil dissolved in 15 ml. of ethyl alcohol and titrating with N/100 sodium hydroxide. The method of Rosenmund and Kuhnhenn (9) was employed to determine the iodine number with the exception that carbon tetrachloride was used to dissolve the sample and the brominating reaction time was exactly 10 minutes. Peroxide values were determined by the ferric thiocyanate method of Lips, Chapman and McFarlane (5).

FLAVOUR JUDGMENTS

FOR every sample of shortening prepared in the course of this investigation the success or failure of the process involved lay in the answer to the question, "Does it give a reverted flavour when used in baking?" Whether or not a sample would give the reverted odour on heating in an oven at 100°C. did not seem to be the real criterion of quality. Although such a test would give an indication of what was probably unsuccessful it would lack finality. Accordingly, all samples were used in baking (generally pastry) and the quality of the shortening was then ascertained by the judgments of the baked products.

Most of the samples were judged qualitatively by four or five judges, but in a number of cases a panel of ten to fifteen judges was organized to quantitatively measure the quality of a particular series of samples. In some cases the flavour and in other cases the odour of the samples was judged. In either case, two methods were employed to assign a numerical score to each sample. In the first method a ten-point scale resembling that of Wilson and Slosberg (10) was used with the points on the scale described briefly in the following manner:

10 Excellent	Considered suitable for household use.
9 Very good	
8 Good	
7 Slight off flavour (odour)	
6 Off flavour (odour)	
5 Slight oily or reverted flavour (odour)	Considered not acceptable for household use.
4 Definite oily or reverted flavour (odour)	
3 Unpleasant flavour (odour)	
2 Very unpleasant flavour (odour)	
1 Repulsive flavour (odour)	

Each sample in a series was scored by each of the judges. The scores for each sample were then averaged. The best score possible was thus 10 and the poorest was 1.

In the second method the judges were asked simply to indicate for each sample in flavour judgment whether or not they considered it of satisfactory flavour for household use; and in the case of odour judgments, whether or not they considered it to have a reverted (or worse) odour. Then to give a rating of the "excellence" of each sample the number of judgments declaring it satisfactory (or not reverted in odour) were added and this total divided by the number of judgments, giving scores in fractional quantities. Thus with 12 judges in a panel, a sample judged satisfactory by all judges would have a score of 12/12 = 1, and a sample judged satisfactory by 6 judges would have a score of 6/12 = 0.5. To have these scores more nearly like those obtained by the first method each was multiplied by 10, thus the two samples mentioned would receive final scores of 10 and 5 respectively. In a number of experiments it was desired to follow the progress of reversion by noting the development of the reverted odour. In these cases it was more convenient to record for each sample the percentage of the judgments which considered it reverted. It should be pointed out that both forms of this second method graded the samples on a scale of eleven points because a very poor sample could receive a score of zero if all judges considered it unsatisfactory.

Experimental

CAUSES OF FLAVOUR REVERSION

A 500-g. sample of alkali-refined linseed oil was saponified and the two fractions separated (3). The nonsaponifiable fraction was added, in the same proportion as it was present in linseed oil, to a sample of alkali-refined sunflower seed oil and to a sample of "blended" shortening, containing up to 65% animal fat. The former mixture was then hydrogenated to the consistency suitable for a shortening and finally deodorized. The two shortenings were used in baking pastry but in neither case was a reverted flavour detected. A portion of the mixed component-fatty acids was re-esterified with glycerol (2), and the resulting neutral oil hydrogenated and used to make pastry. This pastry had the characteristic reverted flavour, thus indicating that flavour reversion was caused by a fatty acid component of the oil and not by the nonsaponifiable matter.

Two samples of deodorized-linseed oil shortenings were found to have acid values (as % oleic acid) of 0.10 and 0.18. A portion of each was alkali-refined, reducing the acid values to 0.028 and 0.031, and the four samples used to prepare pastries. As there were no appreciable differences in the pastries it seemed that the free fatty acids in the shortening were not responsible for reversion.

A sample of ethyl linolenate (B. P. 194°C. at 5.5 mm.) was prepared from linseed oil (7). One portion was blended with sunflower seed oil in the proportions of 40:60 and the mixture hydrogenated. Pastry prepared with this shortening had the characteristic reverted flavour. Another portion was blended with a sunflower seed shortening in the same proportions. The pastry prepared with this blended shortening had an unpleasant flavour but not that characteristic of reversion. It was thus indicated that linolenic acid did cause the reverted flavour, but only after hydrogenation. In this experiment, the flavour imparted by ethyl linolenate itself, could have been misleading. However, we believe this is unlikely as the flavour judgments on the baked products were made by a panel of experienced judges.

MODIFICATIONS IN PROCESSING

Variations in a number of steps in the established methods of processing linseed oil into an edible shortening were studied as follows:

1. Samples of a crude hot pressed linseed oil were hydrogenated to iodine numbers of about 65 with catalyst concentrations varying from 0.4 to 1.7% nickel.

2. Samples of an alkali refined hot-pressed linseed oil were hydrogenated to iodine numbers of about 65 with catalyst concentrations varying from 0.3 to 1.6% nickel.

3. Five samples of un alkali-refined hot-pressed linseed oil were hydrogenated to iodine numbers of about 65 at temperatures of 50, 80, 120, 150 and 180°C.

4. Five samples of a hot-pressed linseed oil and five samples of a cold-pressed linseed oil were refined as follows: (a) no refining, (b) alkali refining, (c) alkali refining, then bisulphite refining, (d) alkali refining, then adsorbent refining with silica gel-alumina, (e) alkali refining, then adsorbent refining with Fitrol-carbon. Each sample was hydrogenated to an iodine number of about 70.

5. Eight samples of a crude hot-pressed linseed oil were hydrogenated to iodine numbers of 61, 67, 72, 76, 83, 87 and 93.

6. Two samples of alkali-refined hot-pressed linseed oil were hydrogenated to iodine numbers of 62 and 68 and blended in the proportions of two and three parts respectively of the linseed oil shortening with one part of alkali-refined sunflower seed oil.

7. Two samples of alkali- and adsorbent-refined (silica gel-alumina) hot-pressed linseed oil were hydrogenated to iodine numbers of 67.5 and 69.0 and blended in the proportions of two and four parts respectively of the linseed oil shortening to one part of sunflower seed oil.

8. Seven samples of linseed oil, including one hot pressed and one cold-pressed oil from each of three seed crushing mills and a sample of a special linseed oil No. 100 from Pittsburgh Plate Glass Co. (iodine number 155) were each divided into four parts, one part hydrogenated directly to an iodine number of 65-70, one part alkali refined and hydrogenated to an iodine number of 65-70 and the other two parts (duplicates) alkali-refined, adsorbent refined and hydrogenated to iodine numbers of 75-80.

All of the above shortenings were subjected to baking tests. From the results it was concluded that an improved shortening, but which still gave a slight characteristic flavour to pastry, was obtained by hardening to an iodine number below 70 and by a greater degree of refining prior to hydrogenation, although bisulphite refining gave no improvement. Hot- or cold-pressed oils gave the same results. Larger amounts of catalyst and higher temperatures reduced the hydrogenating time but had little effect on the quality of the product except in the case of crude oil where an increase in catalyst concentration did bring some improvement although this may have been due entirely to the silica gel partially refining the oil by adsorption. The experiments with blends of sunflower seed oil and hydrogenated linseed oil showed that a shortening with desired physical characteristics could be prepared and its presence in fresh or toasted slices of bread could not be detected when used at a level of 4% of the dough. However, the flavour could still be detected when pastry was prepared with these shortenings

SPECIAL TREATMENTS AND TECHNIQUES

Conjugated Hydrogenation: Two samples of alkali-refined hot-pressed linseed oil were hydrogenated in a pyrex flask in the presence of 1% nickel catalyst at 200°C. while vapours of ethyl alcohol passed continuously through the mixture at approximately atmospheric pressure. There was no apparent difference in the course of the hydrogenation when absolute or 95% alcohol was used, the iodine number being 120 at 3 hours and 100 at 12 hours. Pastry prepared from these shortenings gave the characteristic reverted flavour.

Isomerization: Attempts to bring about isomerization following the hydrogenation of alkali-refined
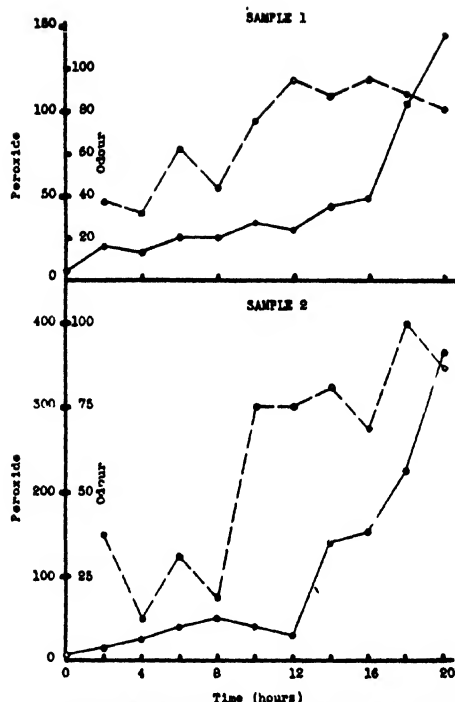
FIG. 1. Showing the relationship between the degree of flavour reversion and the peroxide content.

————○———— Peroxide-oxygen (milliequivalents per Kg.)

— — —○— — — Odour (% of judgments which indicated the sample was reverted)

hot-pressed linseed oil involved the following experiments: (a) heating at 230°C. for 12 hours in an atmosphere of hydrogen using the hydrogenation apparatus to agitate the oil; (b) heating at 230-240°C. for 6 hours with sulphur dioxide bubbled through at a rate sufficient to keep the mixture agitated, and (c) heating at 230-240°C. for 6 hours with one percent selenium using a mechanical stirrer to agitate the mixture The quality of the shortening was not improved by any of these treatments.

Oxidation Before and After Hydrogenation: A sample of alkali-refined linseed oil was heated for 12 hours at 98-100°C. while agitated with a current of air. This slightly oxidized oil was hydrogenated to an iodine number of 71. Pastry prepared from this product had a more objectionable flavour than the characteristic reverted flavour. A sample of alkali-refined linseed oil was hydrogenated to an iodine number of 69.5, divided into two portions, and air blown through one portion at 150-160°C. for 6 hours and through the other at 180-190°C. for 8 hours. These treatments did not improve the shortening.

However, we have observed that the degree of flavour reversion parallels the increase in peroxide-oxygen during the oxidation of linseed shortenings. The formation of peroxides and the development of reverted odour were followed in two samples of hydrogenated linseed shortenings stored at 100°C. for 20 hours. The first appearance of reverted odour and the rate at which it increased were determined by a panel of judges. The relationship between peroxide-

oxygen and reverted odour was found to be essentially the same for both samples. The data presented in Figure 1 indicates that the first appearance of the reverted odour does not coincide with the end of the induction period as detected by peroxide values, but rather that the onset of flavour reversion precedes the point at which there is a marked increase in peroxides.

Fractionation with Acetone: Three samples of alkali-refined linseed shortening of iodine numbers 83, 65 and 54 respectively were fractionated by crystallization from acetone (3) giving three fractions, viz., (I) crystallized at room temperature, (II) soluble at room temperature but crystallized at —15°C., (III) soluble at —15°C. The yields and the iodine numbers of each fraction from the three shortenings are shown in Table I.

TABLE I
Fractionation of Linseed Shortening From Acetone

	Acetone Fractions		
	Fraction No.	Yield (%)	Iodine Number
Shortening No. 1, Iodine Number-83	I	14.0	58
	II	58.7	86
	III	27.3	112
Shortening No. 2, Iodine Number 65	I	39.8	46
	II	45.6	81
	III	14.8	95
Shortening No. 3, Iodine Number 54	I	55.0	39
	II	33.8	74
	III	11.8	87

The development of peroxides and of reverted odour at 100°C. in each of the three fractions of each shortening was studied with the results shown in Figure 2. These results confirm the previous observations that the onset of flavour reversion is accompanied by an increase in peroxide-oxygen. It will be observed that all of the samples showed flavour reversion but in varying degrees, fraction I being the most stable. However, it was expected from the relative iodine numbers that there would be a greater difference in stability between fractions II and III. It is of interest to note that in each case fraction I showed a marked increase in peroxides before the appearance of the reverted odour, whereas in this respect fractions II and III behaved in a manner typical of the unfractionated shortening (see Fig. 1).

The Use of Antioxidants: Since there appeared to be a relation between flavour reversion and oxidation in linseed shortenings, experiments were next carried out to determine whether antioxidants, known to retard the formation of peroxides in fats, would also retard or prevent flavour reversion in these shortenings. Figure 3 presents the results obtained with "Viobin Antioxidant" (6) alone, and supplemented with isopropyl gallate (1). The antioxidants were added to the deodorized linseed shortening because, in this case, the activity of the antioxidant is greatly reduced if it is added at any earlier stage in proc-
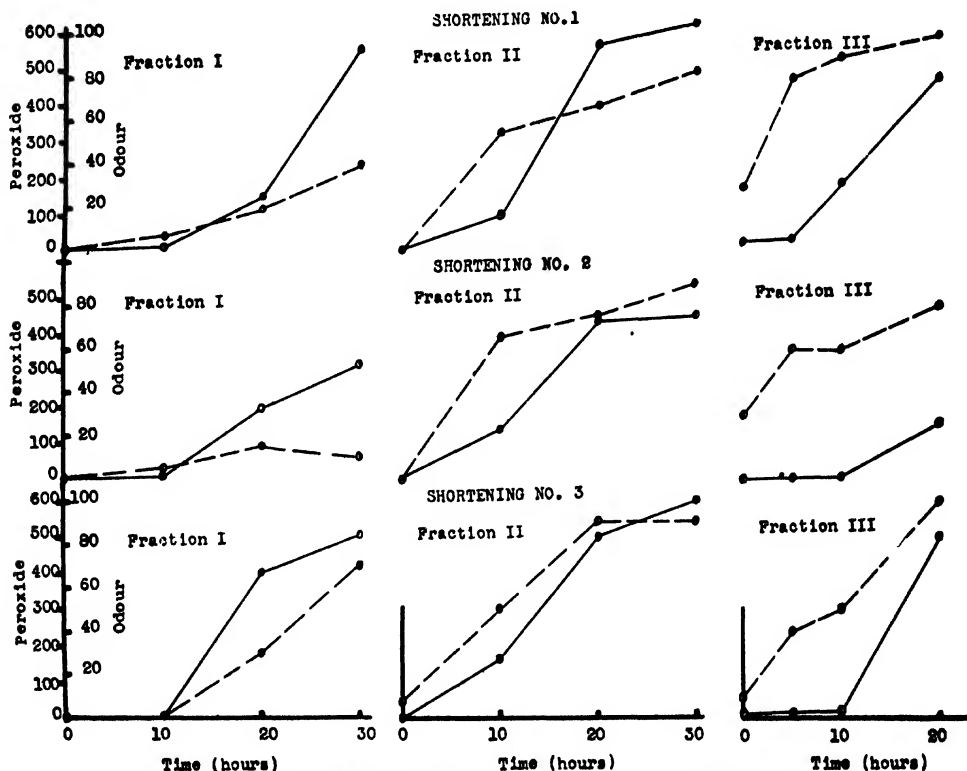

Fig. 2. Showing flavour reversion and peroxide content of fractions crystallized from acetone.
————————⊙———————— Peroxide-oxygen (milliequivalents per Kg.)
——— ——— ⊙——— ——— Odour (% of judgments indicating sample reverted)

[4]

ening. It is evident from the results presented that the antioxidants have retarded the formation of peroxides and of the reverted odour. When these samples of shortening were subjected to baking tests some improvement in flavour was noted but the characteristic reverted odour was detectible in every case.

These findings were confirmed in further experiments in which many other commonly used antioxidants were tested singly or in combination and at different levels. It was therefore concluded that antioxidants even at relatively high levels did not entirely prevent the development of a reverted flavour during baking tests.

General Discussion

THE results of this investigation support the theory, developed by Lemon (4), that linolenic acid is responsible for flavour reversion in linseed shortenings. However, the characteristic reverted flavour seems to arise, not from this substance itself, either as a free fatty acid or in glyceride linkage, but from some product formed from it during hydrogenation. Regarding the nature of this product it can only be said that it isn't a free acid and that it does not seem to be offensive in itself, but on heating it produces the substances which are directly responsible for the flavour and odour. These latter substances may be removed by deodorization whereas the basic cause may not; it remains and is able, on subsequent heating to form again the odoriferous substances

In the work here completed the identification of this substance has not been attempted. There are many possibilities, only a few of which can be eliminated: the ordinary oleic, linoleic and linolenic acids are ruled out, likewise the usually occurring iso-oleic acids cannot be responsible. If linolenic acid were first hydrogenated at the $\triangle^{15,16}$ double bond the ordinary linoleic acid would be obtained and no reversion should occur thereafter. Therefore, at least a part of the linolenic acid must have undergone some other change, which may involve reduction at one of the other double bonds or an isomerization reaction, either of these may be followed by the other and the isomerization may involve migration of the double bonds of cis-trans changes. The possibilities thus created are many and the available methods of study may not be adequate to give a definite answer.

Regarding the reversion some possible solutions may be offered. There is a possibility that hydrogenating conditions may be made so highly selective that all the linolenic acid would be changed into ordinary linoleic before any other change occurred, or that some change, e.g., oxidation, might be made to occur in the original linolenic acid so that on hydrogenation the reaction would take a more favourable course. Also the hydrogenated product might be changed through a subsequent isomerization to restore the structure to one which is heat-stable and inoffensive, or through a treatment such as prolonged heating (and possibly oxidation) accompanied by deodorization the basic substance might be converted into volatile products which would be removed, or finally certain substances might act as inhibitors to prevent the usual decomposition during baking.

The experiments here described have attempted to investigate these possible solutions but no satisfactory method can be claimed.
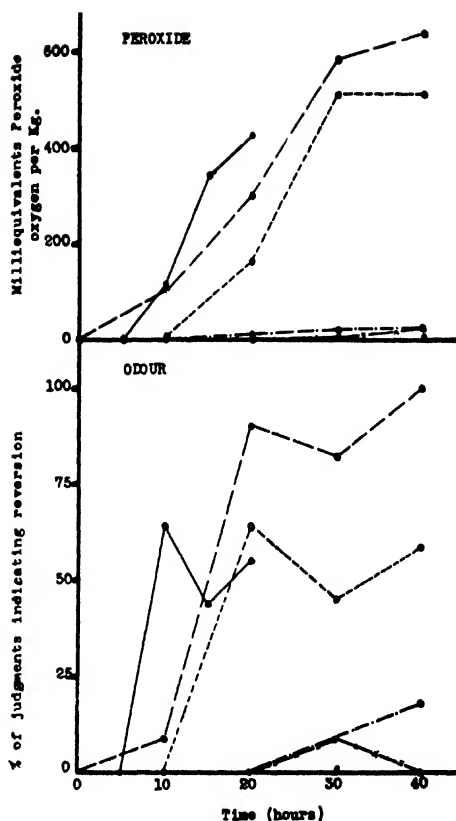
FIG. 3. Showing the effect of antioxidants on flavour reversion and peroxide formation in linseed shortenings at 100°C.

————————⊙———————— Control—No antioxidant
— — — —⊙— — — — 0.01% Viobin antioxidant
– – – – –⊙– – – – – 0.01% Viobin antioxidant +0 0025% isopropyl gallate
———————⊙—·——·— 0.10% Viobin antioxidant
—x—x—⊙—x—x— 0.10% Viobin antioxidant +0 025% isopropyl gallate

Summary

1. The production of an edible shortening by the hydrogenation of linseed oil with dry-reduced nickel catalyst has been studied.

2. A derivative of linolenic acid formed during hydrogenation, is shown to be responsible for flavour reversion in linseed oil shortening. It is believed that this substance decomposes to form the volatile products directly responsible for the reverted flavour and odour.

3. Possibilities of producing linseed oil shortening free of reversion have been investigated as to (a) pretreatment of the oil, (b) changing hydrogenation conditions, (c) changing the hydrogenation product by isomerization, (d) destruction of the offensive product of hydrogenation and removal by deodorization, and (e) the use of antioxidants to inhibit reversion in the hydrogenated product.

4. Improved products have been obtained but flavour reversion has never been entirely eliminated.

[5]

Acknowledgments

One of us (J. G. A.) is the recipient of a Fellowship from the Swift Canadian Company which is gratefully acknowledged.

In this investigation we have collaborated with the section on fats of the Canadian Committee on Food Preservation, and we are indebted to members of this Committee for suggestions in the planning of this research and for supplying some of the samples.

BIBLIOGRAPHY

1. Bergel, P., Chem. and Indus. *62*, 406-7 (1945).
2. Garner, T. L., J. Soc. Chem. Ind. *47*, 275-80T (1928).
3. Hilditch, T. P., "The Chemical Constitution of Natural Fats." John Wiley and Sons, Inc., New York (1940).
4. Lemon, W. H., "Flavour reversion in hydrogenated linseed oil. I. The production of an isomer of linoleic acid from linolenic acid." Can. J. of Res., in press.
5. Lips, A., Chapman, R. A., and McFarlane, W. D., Oil and Soap *22*, 240-3 (1945).
6. Lips, A., and McFarlane, W. D., Oil and Soap *10*, 193-6 (1943)
7. McCutcheon, J. W., Organic Synthesis *22*, 82-6 (1942).
8. Methods of Analysis, A.O.A.C., 4th Edition, (1935).
9. Rosenmund, K. W., and Kuhnhenn, W. Z., Untersuch, Lebensm. *46*, 154-9 (1923).
10. Wilson, R. V., and Slosberg, H. M., Food Industries *14*, 56-8 (1943).

DRIED WHOLE EGG POWDER

XV. THE GROWTH OF *SALMONELLA* AND OTHER ORGANISMS IN LIQUID AND RECONSTITUTED EGG[1]

By N. E. Gibbons[2], R. L. Moore[3], and C. O. Fulton[2]

Abstract

Curves are presented showing the growth in liquid egg of *Salmonella bareilly*, *S. manhattan*, *S. typhi-murium*, *S. oranienburg*, *S. typhi*, *Escherichia coli*, *Aerobacter aerogenes*, *Staphylococcus aureus*, *Streptococcus fecalis* and *S. pyogenes*, and of *Salmonella bareilly* in reconstituted egg. *Streptococcus pyogenes* does not grow in egg and dies off rapidly at temperatures above 60° F. (15.6° C.). The other organisms generally grow well in liquid egg at temperatures above 60° F. (15 6° C.). Liquid and reconstituted egg should therefore be maintained well below this temperature to prevent the multiplication of *Salmonella* and other possible pathogens.

Introduction

Organisms of the *Salmonella* group have been reported in dried egg powder (1, 2, 6) but the number is usually small (2). Although drying kills most of the organisms, the greater the number of organisms in the liquid egg the greater the number likely to survive the drying process (3). Since these organisms may multiply in the reconstituted egg and form a health hazard, it was of interest to determine at what temperatures growth may occur in liquid and reconstituted egg. *Staphylococcus aureus* and *Streptococcus pyogenes*, which are also of public health significance, as well as other organisms commonly present on eggs, were included in the study.

Methods

To simplify the task of making counts, sterile egg melange was used. Eggs, one day old, were used for most of the work, although commercial Grade A eggs were found to yield a high percentage of sterile meats. The method used to obtain sterile meats was essentially that of Rosser (4). One egg is allowed to soak in a 500 p.p.m. chlorine solution while the previously treated egg is being opened. The egg is held between two small ring clamps fastened to a bar, bent at right angles, which is in turn fastened to a ring stand so that the egg may be inverted by loosening the clamp at the ring stand (Fig. 1, A). The large end of the egg is painted with tincture of iodine and a triangular piece of shell cut out with a sterile abrasive wheel in an electric hand drill. The shell and outer membranes are removed with sterile forceps. The egg is then inverted, the small end painted with iodine, and a small hole made in it with the drill. The inner membrane at each end is pierced with a platinum

[2] *Bacteriologist, Food Investigations.*

[3] *Laboratory Assistant, Food Investigations.*

wire and the egg contents blown into a sterile wide mouthed flask by means of compressed air filtered through a Seitz filter (Fig. 1, B). The flasks, each containing one egg, are incubated overnight and checked the next day for growth by subculturing. The sterile eggs are then poured into a large Seitz filter fitted with a fine wire screen in place of the filter disk, and the egg is forced through the screen by compressed air (Fig. 1, C). The egg is caught in a delivery bottle, mixed, dispensed into sterile flasks as needed and again checked for sterility. Using this method sterile meats were obtained from 80 to 95% of Grade A commercial eggs.

Most of the organisms used in this study were isolated from egg powder: *Salmonella bareilly; S. manhattan; S. oranienburg; S. typhi-murium; Escherichia coli; Aerobacter aerogenes;* and *Streptococcus fecalis.* The other organisms used were: *Salmonella typhi,* Hopkins disinfectant strain; *Staphylococcus aureus,* Wood 46, a non-enterotoxin producing strain; and four strains of *Streptococcus pyogenes,* Group A* (Matthews Type 25 and three strains isolated from scarlet fever patients, Types 1, 4, 12-14).

Growth was followed by the procedure outlined below. The melange was inoculated with 24-hr. broth cultures of the organisms, distributed into the necessary number of flasks and incubated. The liquid egg was heated rapidly to the desired temperature in a water-bath before being placed in the incubator. In the first experiments the temperatures used were 98.6°, 86°, 75°, 68°, 60°, 52°, and 45° F. (37°, 30°, 24°, 20°, 15.6°, 10.6°, and 7.2° C.); in later experiments 90°, 80°, 70°, 60°, 50°, and 40° F. (32.2°, 26.7°, 21.1°, 15.6°, 10.0°, and 4.4° C.). Aliquots were removed at 6, 12, and 24 hr. at the higher temperatures and also at 48 hr. at the lower temperatures since under practical conditions liquid or reconstituted egg would not likely be held for longer periods. Appropriate dilutions were plated on proteose-peptone tryptone agar. The streptococci were plated on heart infusion agar. All plates were incubated at 98.6° F. and counted after three days.

Since it is practically impossible to obtain sterile spray dried egg powder, growth curves in reconstituted egg are difficult to obtain. Curves for *Salmonella bareilly* were obtained by using powder free of other sulphide-producing organisms and following growth by the most probable number technique previously described (2).

Results

The growth curves of *Salmonella* in liquid egg are shown in Fig. 2 (A and B) and Fig. 3 (A, C, and D). In each there is a decided break in the rate of growth around 60° F. (15.6° C.). Above this the rate increases with increasing temperature to about 86° F. Below 60° F. there is little or no increase and in some instances a decrease. Only one strain (*S. manhattan,* Fig. 2, B) showed rapid growth at 60° F.: in all others there was a definite lag of at least 12 hr. followed by a fairly rapid increase. For practical purposes it is evident that if the egg liquid is kept below 60° F. there is little

---

* *Kindly supplied by Dr. E. T. Bynoe, Laboratory of Hygiene, Department of Pensions and National Health, Ottawa.*

PLATE I
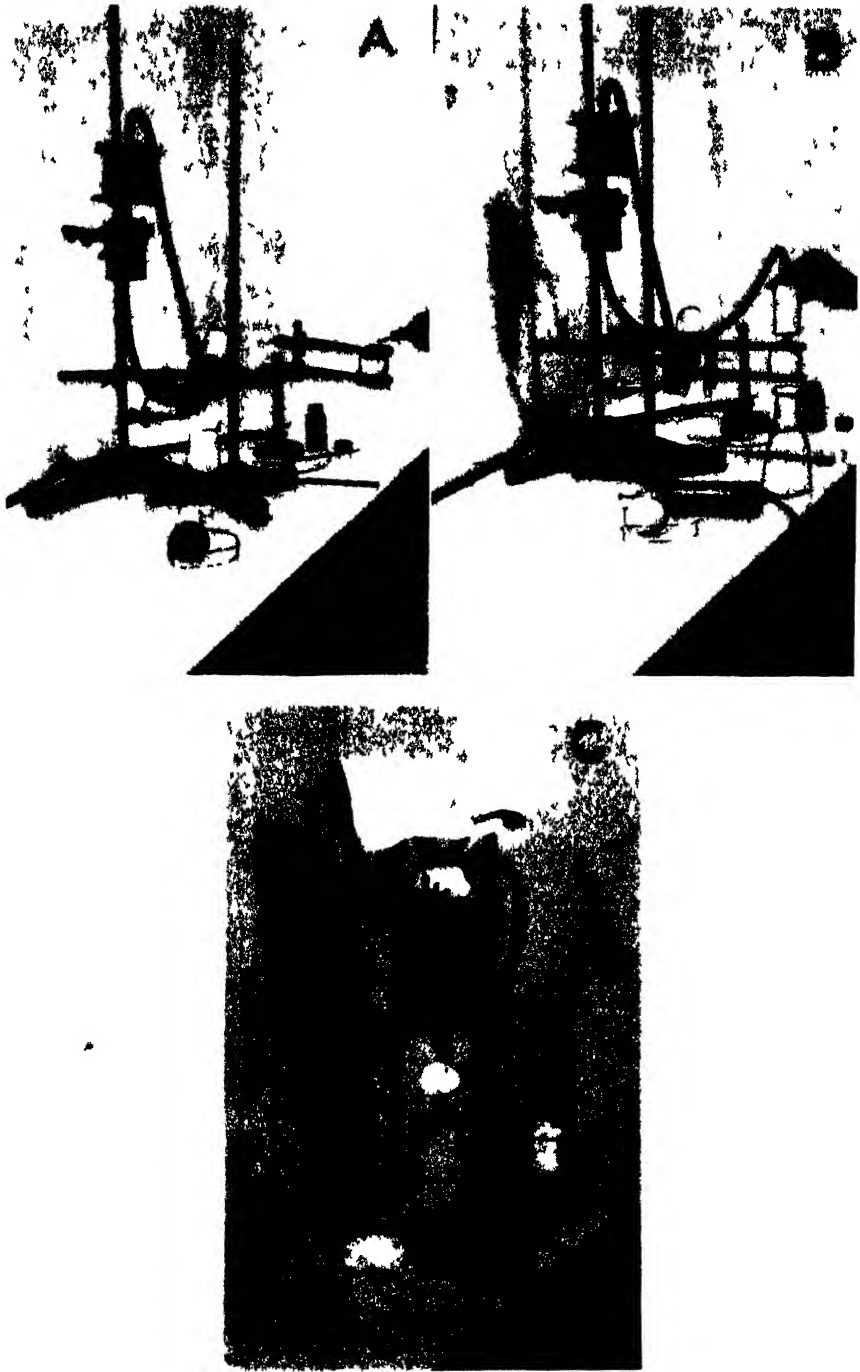
Fig 1 *Apparatus for obtaining sterile egg meat* A *Cutting the shell.* B *Blowing out the contents* C *Seitz filter and apparatus for straining and dispensing liquid egg*
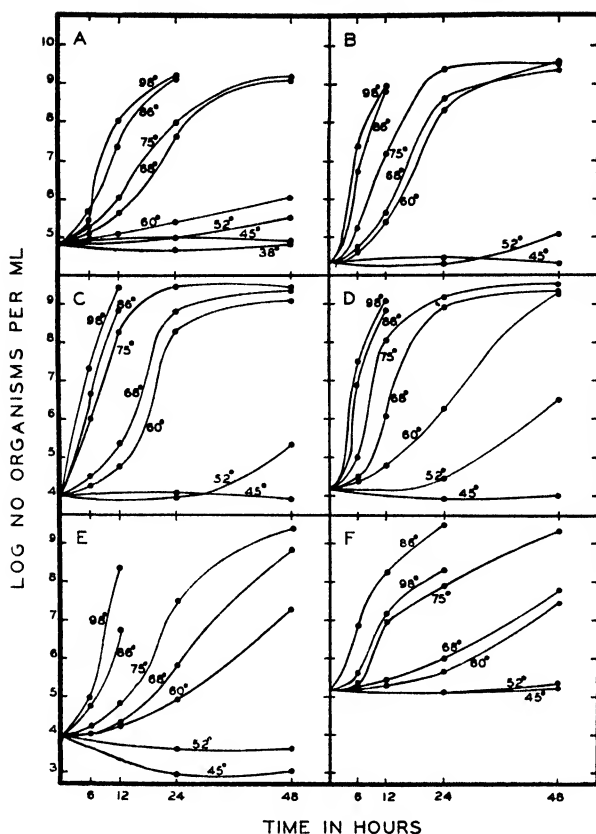
FIG. 2. *Growth curves in liquid egg at 98°, 86°, 75°, 68°, 60°, 52°, and 45° F. of:* A,—*Salmonella bareilly;* B,—*S. manhattan;* C,—*E. coli;* D,—*A aerogenes;* E,—*S. typhi; and* F,—*Staphylococcus aureus.*

danger of multiplication of *Salmonella* organisms over the period studied. At room temperature (68° F.) there is a fairly rapid growth and the final number is at least as great in 48 hr. as at the higher temperatures. This agrees with work recently reported for canned foods (5).

Salmonella bareilly does not appear to grow quite as well in reconstituted egg (Fig. 3, B) as in liquid egg (Fig. 3, A). This difference may be due in part to the different method of estimating the number of organisms. Although there is a definite lag period the original decreases at 60° F. and 70° F. are possibly fortuitous.

The growth of *E. coli* and *A. aerogenes* (Fig. 2, C and D) is essentially the same at the higher temperatures. The strain of *A. aerogenes* used is not affected by the lower temperatures in the same way as *E. coli*. Below 60° F. *Salmonella typhi* (Fig. 2, E) decreased in numbers over the period studied.

Staphylococcus aureus (Fig. 2, F) was the only organism used that grew more rapidly at 86° than at 98° F. It, however, does not grow as well at room temperatures as the *Salmonella* strains.
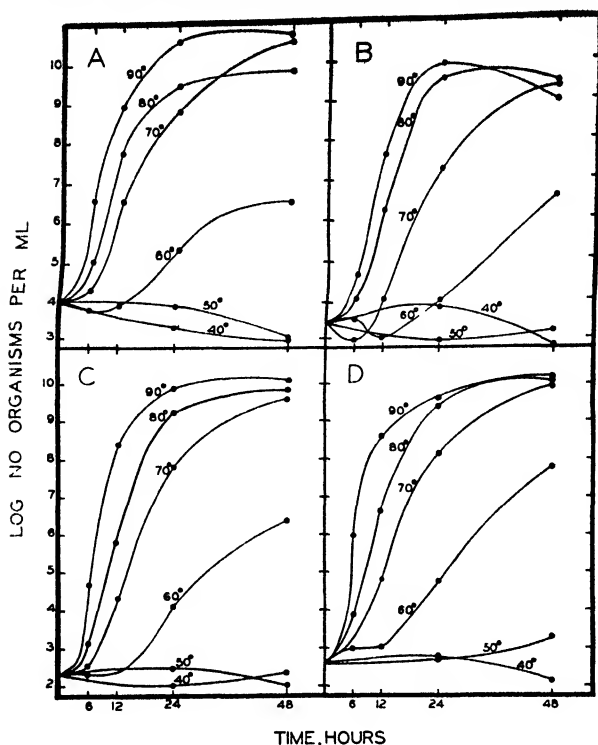
FIG. 3. *Growth curves of Salmonella bareilly in: A,—egg liquid; and B,—reconstituted egg powder; and of C,—S. typhi-murium and D,—S. oranienburg, in liquid egg. Temperatures in ° F.*
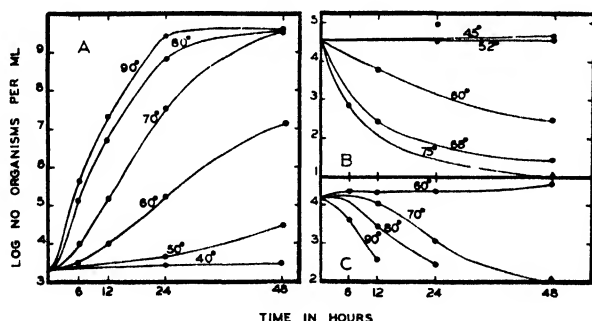
FIG. 4. *Growth curves in liquid egg of: A,—Streptococcus fecalis; B,—S pyogenes (Matthews strain); and C,—S pyogenes, Group A. Temperatures in ° F.*

Streptococcus fecalis does not grow as rapidly at the higher temperatures as the majority of other organisms (Fig. 4, A). None of the strains of *Streptococcus pyogenes* grew in liquid egg. They survive at the lower temperatures but die off rapidly above 60° F. The curves obtained for two strains are presented (Fig. 4, B and C). Similar curves were obtained for the other two strains.

Summary

Group A streptococci do not grow in liquid egg. This is probably one explanation of the fact that they are not usually found in egg powder*.

For the other organisms studied, 60° F. seems to be the dividing line between rapid growth and little or no growth over a 48 hr. period. At this temperature, there is usually a lag period of about 12 hr., which may or may not be followed by rapid growth. In actual drying practice, where liquid egg is seldom held longer than 12 hr. and usually at temperatures of 45° F., there would be little danger of multiplication. However, if reconstituted egg were allowed to stand overnight at room temperature multiplication would take place.

References

1. BORNSTEIN, S., SAPHRA, I., and STRAUSS, L.   J. Infectious Diseases, 69 : 59-64.   1941.
2. GIBBONS, N. E. and MOORE, R. L.   Can. J. Research, F, 22 : 48-57.   1944.
3. GIBBONS, N. E. and MOORE, R. L.   Can. J. Research, F, 22 : 58-63.   1944.
4. ROSSER, F. T.   Can. J. Research, D, 20 : 291-296.   1942.
5. SEGALOVE, M. and DACK, G. M.   Food Research, 9 : 1-5.   1944.
6. SELIGMANN, E., SAPHRA, I., and WASSERMANN, M.   Am. J. Hyg. 38 : 226-249.   1943.

Unpublished data.

FLAVOUR REVERSION IN HYDROGENATED LINSEED OIL

I. THE PRODUCTION OF AN ISOMER OF LINOLEIC ACID FROM LINOLENIC ACID[1]

By H. W. Lemon[2]

Abstract

Linseed oil that has been hydrogenated to a plastic consistency is subject to a type of deterioration termed "flavour reversion" when heated to temperatures used in baking or frying. Investigation of the course of hydrogenation of linseed oil by the spectral method of Mitchell, Kraybill, and Zscheile (11) has indicated that linolenic acid is converted to an isomeric linoleic acid; this acid differs from naturally occurring linoleic acid in that the double bonds are in such positions that diene conjugation is not produced by high-temperature saponification. In a typical hydrogenation, the concentration of the isomeric acid increased to a maximum, at about iodine number 120, of 18% of the total fatty acids, and at iodine number 80, at which point the plasticity was similar to that of a commercial shortening, the concentration of the isomer was 13%. Evidence is presented that the isomeric linoleic acid in partially hydrogenated linseed oil is responsible for the unpleasant flavour that develops when the oil is heated.

The shortage of vegetable oils that developed in Canada after the outbreak of war in the Pacific has made it advisable to investigate the possibility of using linseed oil in the manufacture of shortenings, as it is the only vegetable oil that is produced in this country in large quantities. Linseed oil can be hydrogenated easily, but it requires considerably more hydrogen than the oils normally used. However, the greatest obstacle in the way of utilizing hydrogenated linseed oil in shortenings is that it is subject to a type of deterioration termed "flavour reversion." When freshly deodorized, it is reasonably bland, but it develops an off-flavour on storage, and a characteristic, unpleasant odour when it is subjected to baking or frying temperatures. In connection with investigations undertaken to determine the effect of different conditions of hydrogenation on flavour reversion, to be published later, some evidence was obtained indicating that a hydrogenation product of linolenic acid contributed to this reversion. This evidence is presented in the present article.

The problem of flavour reversion is well known in the edible oil industries. Fish oils and soybean oil are subject to it, but to a lesser extent than linseed oil. Flavour reversion in soybean oil has been ably discussed by Bickford (3), and he has reviewed the theories that have been proposed to explain the phenomenon. Some evidence has been put forth that a non-glyceride constituent of the oil is the cause of the trouble. Others (7) suggest that linolenic acid is in some way responsible for it, and the fact that the tendency to flavour reversion in linseed and soybean oils is roughly proportional to their linolenic acid content lends some support to this theory.

It has been suggested that the development of an unpleasant flavour in partially hydrogenated linseed oil may be due to linolenic acid that has escaped hydrogenation. The glycerides of linseed oil contain 30 to 60% of linolenic acid and 10 to 25% of linoleic acid. When an oil is hydrogenated, the rise in melting point is due to the formation of both saturated fatty acids and "iso-oleic acid," which is a solid isomer of oleic acid. If the reaction is selective, the most unsaturated fatty acids are largely converted to less unsaturated acids before the latter become completely hydrogenated. Selectivity is influenced by the operating variables, but, as Bailey, Feuge, and Smith (1) have pointed out, the formation of iso-oleic acid is favoured by each of the conditions that contribute to selectivity. Therefore, linseed oil, since it contains such a large proportion of linolenic and linoleic acids, may become too hard on hydrogenation before these highly unsaturated acids have disappeared. It was decided that the change in fatty acid composition that takes place as hydrogenation proceeds should be accurately determined, with the hope that it might provide a clue to the cause of flavour reversion.

Experimental

Procedure

Raw linseed oil was refined with an aqueous solution of sodium hydroxide, containing $4\frac{1}{2}$ times the quantity of alkali required for neutralization of the free fatty acids, then bleached with an activated earth. The refined oil was hydrogenated in a vertical cylindrical steel pressure vessel, with a capacity of about 2 lb. of oil, which was equipped with an agitator of the paddle type, cooling coil, and thermometer well. Electrolytic hydrogen was supplied from a cylinder through a pressure regulating valve and a tube extending through the top of the vessel into the oil. Heat was supplied by means of a ring gas burner underneath the vessel. A variety of temperatures and pressures have been employed, and both commercial and laboratory prepared catalysts were used. The laboratory catalysts were of two types, (*a*) nickel formate reduced in oil at 265° C. in an atmosphere of hydrogen, and (*b*) nickel carbonate reduced dry at 400° C. in an atmosphere of hydrogen. After the conclusion of each hydrogenation, the catalyst was removed by filtration, and the hydrogenated oil was deodorized with steam for three hours at 200° C. and a pressure of 2 to 4 mm. of mercury.

Methods of Analysis

The Kaufmann (10) method of analysis was first applied to hydrogenated linseed oil samples. This method entails the determination of the iodine and thiocyanogen numbers, and the saturated acid content of the sample, and from these values oleic, linoleic, and linolenic acid percentages are calculated. The fatty acid composition of various linseed oils has been determined by Rose and Jamieson (15) and by Painter and Nesbitt (12) in this way. Similar results were obtained for the linseed oil used in the hydrogenation experiments, but when the method was applied to hydrogenated linseed oil, the calculated values for the unsaturated fatty acids were impossible, as the linoleic acid

values were consistently negative over a wide range of iodine numbers when the constants suggested by Riemenschneider, Swift, and Sando (13) were used. The results indicated that some product of the hydrogenation did not absorb thiocyanogen in the expected manner.

A new method of fat analysis has been developed by Mitchell, Kraybill, and Zscheile (11) that utilizes the fact that fatty acids containing conjugated double bonds show characteristic absorption bands in the ultra-violet region of the spectrum. Conjugation is achieved by saponification of the fat sample at 180° C. with a potassium-hydroxide–ethylene-glycol solution. Conjugated linoleic acid has an absorption maximum at 2340 Å, while conjugated linolenic acid has maxima at 2340 Å and at 2680 Å and smaller maxima at 2580 Å and 2790 Å. The specific absorption at 2340 Å and 2680 Å has been determined for the pure acids with the use of a spectrophotometer; the values are reproducible when the alkali treatment is standardized. If the specific absorption at 2340 Å and 2680 Å of the sample to be analysed is determined, the concentration of linolenic and linoleic acids can be calculated by comparison of the absorption with that of the pure acids. Very small quantities of linolenic acid can be detected and measured in this way.

The spectral method of analysis has been applied to hydrogenated linseed oil, in conjunction with the determination of saturated and iso-oleic acids by the lead salt precipitation method of Baughman and Jamieson (2). A Beckman spectrophotometer (6) was used for the absorption measurements.

Results

Results of Spectral Analysis

The specific absorption measurements at 2680 Å showed that linolenic acid disappeared rapidly on hydrogenation of linseed oil. It was expected that there would be a corresponding increase in linoleic acid during the initial stages of hydrogenation, but absorption measurements at 2340 Å indicated, on the contrary, that the linoleic acid content became steadily less.

The percentages of oleic and saturated acids were calculated from the iodine numbers of the samples and from the values for linoleic and linolenic acids that were obtained by spectral analysis. The calculated oleic acid values were much higher than is usual in hydrogenated oils, and the values for saturated acids were considerably lower than those arrived at by the lead salt method. As an example, a hydrogenated linseed oil with iodine number 88 contained 2.4% linolenic acid and 2.7% linoleic acid, as determined by spectral analysis; total oleic acid was calculated to be 89.8% and saturated acids 5.1%. Such values are highly improbable, as the value for saturated acids obtained by the lead salt method was 20.5%.

Linoleic and oleic acid percentages can be calculated if the iodine number of the sample, and the values for saturated, iso-oleic and linolenic acids, as determined by the lead salt method and by spectral analysis, are known. When this was done for the above sample, the linoleic and oleic acid values were 17.8 and 44.2%, respectively. It was suspected that the reason for the

difference between the calculated value for linoleic acid and that arrived at by spectral analysis is that hydrogenation of linolenic acid produces an isomeric linoleic acid in which the double bonds are in such a position that a conjugated system is not formed on treatment with potassium hydroxide at 180° C.

Isolation of Isomeric Linoleic Acid

In order to prove the presence of an isomeric linoleic acid in partially hydrogenated linseed oil, a quantity of the separated fatty acids of iodine number 82.1 was dissolved in acetone, and subjected to the low temperature crystallization method for purification of linoleic acid described by Brown *et al.* (4, 5, 8, 9). Most of the saturated and oleic acids crystallized, and were removed by inverted suction filtration. Approximately 12% of the original acids remained in solution, and, after removal of the acetone, the residue was distilled *in vacuo*. The iodine number of the distillate was 152.1 and, if it is assumed that it is a binary mixture of oleic and linoleic acids, the concentration of the latter would be 68%.

Fatty acids separated from sunflower seed oil of iodine number 134.0 were treated in a similar manner to obtain a natural linoleic acid concentrate for comparison with that separated from hydrogenated linseed oil. The distilled concentrate had an iodine number of 162.6, corresponding to a linoleic acid concentration of 80%.

The two fractions were heated with the ethylene-glycol–potassium-hydroxide reagent of Mitchell, Kraybill, and Zscheile, and spectral absorption measurements were made on the soap dissolved in absolute alcohol. Absorption is expressed as "specific alpha."

$$\text{Specific } \alpha = \frac{\log_{10}\dfrac{I_0}{I}}{cl}$$

where α = absorption coefficient,

I_0 = intensity of radiation transmitted by the solvent,

I = intensity of radiation transmitted by the solution,

c = concentration of solute in grams per 1000 ml.,

l = length in centimetres of solution through which the radiation passes.

The absorption curves for the linoleic acid concentrates from sunflower seed oil and from hydrogenated linseed oil are shown in Fig. 1, Graph *A*. Curve 1 for natural linoleic acid shows the characteristic absorption maximum at 2340 Å due to diene conjugation, with a specific α value of 76.7. Mitchell, Kraybill, and Zscheile found the specific α value for pure linoleic acid to be 87.1. In comparison, the absorption at 2340 Å due to the linoleic acid concentrate from hydrogenated linseed oil, as shown in Curve 2, is small. Curve 2 has been replotted in Graph *B* with the specific α scale expanded 10 times in order to show more clearly the maximum in the region of 2340 Å, and
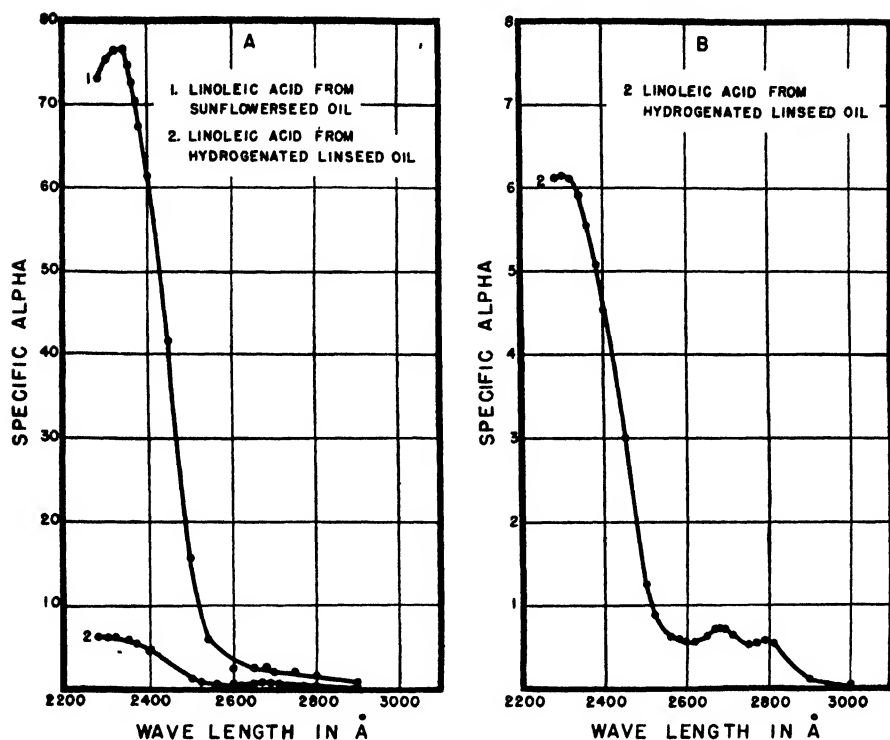
FIG. 1. *Absorption spectra of linoleic acid concentrates from sunflower seed oil and from hydrogenated linseed oil. Graph B is a replot on an enlarged scale of Curve 2 of Graph A.*

smaller maxima at 2680 Å and 2790 Å. The maximum near 2340 Å is probably caused by the presence in the distilled concentrate of a small amount of linoleic acid from the linseed oil that has escaped hydrogenation. The maxima at 2680 Å and 2790 Å are typical of triene conjugation, and indicate the p esence of a trace of linolenic acid in the concentrate.

These results prove beyond doubt that hydrogenation of linolenic acid causes the formation of an isomeric linoleic acid that differs from natural linoleic acid in that the double bonds are so arranged that they will not form a conjugated system.

The Course of Hydrogenation of Linseed Oil

The change in fatty acid composition that occurs during a typical linseed oil hydrogenation is shown graphically in Fig. 2. The oil was hydrogenated at 140° C., 25 lb. gauge pressure, using an oil-reduced nickel formate catalyst in a concentration equivalent to 0.2% of nickel, based on the weight of oil. Samples were taken periodically. Saturated acids and iso-oleic acid were determined by the Baughman and Jamieson method, linolenic and linoleic acids by spectral analysis. Oleic and total linoleic acids were calculated in the way already described, and the values for isomeric linoleic acid (this acid will be termed "iso-linoleic acid") were obtained by subtracting the
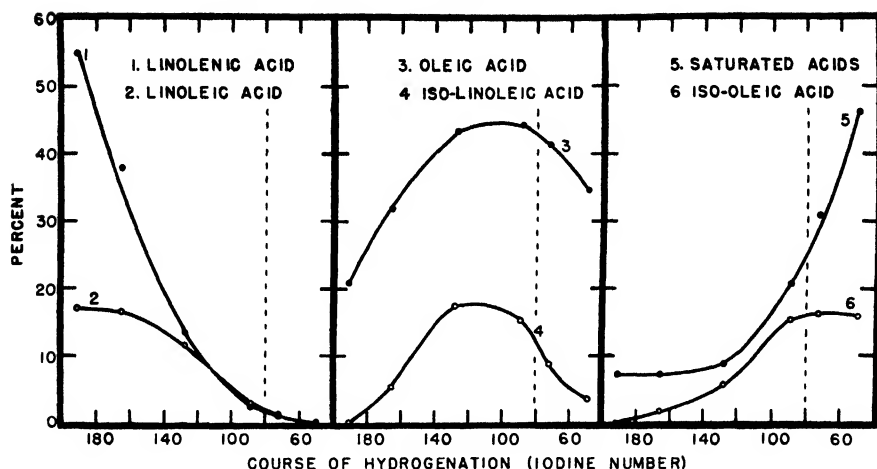
FIG. 2. *Progressive change in fatty acid composition on hydrogenation of linseed oil. The broken vertical lines at iodine number 80 mark the point at which the consistency of the oil was similar to that of a shortening.*

figures for linoleic acid indicated by spectral absorption from the calculated values for total linoleic acid.

The hydrogenation of the linolenic and linoleic acid fractions of the linseed oil was initially rapid, and was accompanied by marked increases in iso-linoleic, oleic, and iso-oleic acids, but by only a small increase in saturated acids. The concentrations of oleic and iso-linoleic acids were greatest at about iodine number 120. Further hydrogenation caused a rapid increase in saturated acids with corresponding decreases in oleic and iso-linoleic acids; during this phase of the reaction linolenic acid was hydrogenated more slowly. Hydrogenated oil of iodine number between 80 and 85 had a melting point similar to that of a commercial shortening, but there still remained traces of linolenic and linoleic acids, and about 14% of iso-linoleic acid.

The fatty acid compositions of a number of partially hydrogenated linseed oils, prepared from the same bulk of linseed oil, are given in Table I. Some of the oils contained traces of linolenic acid, particularly those hydrogenated at low temperature. All the samples contained considerable quantities of iso-linoleic acid. The analyses of Samples 105, 99, and 110 show the effect of hydrogenation temperature on the fatty acid composition. Increasing the temperature caused a decrease in the production of saturated acids, and an increase in iso-oleic acid. The effect of temperature increase on the iso-linoleic acid content of the hydrogenated oil was not very great.

Role of Iso-linoleic Acid in Flavour Reversion

It was suspected that the iso-linoleic acid content of a hydrogenated linseed oil shortening may be responsible for the characteristic and unpleasant flavour that develops in the shortening when it is heated. Strong, though

TABLE I

FATTY ACID COMPOSITION OF HYDROGENATED LINSEED OIL SAMPLES

Number	Temperature of hydro-genation, °C.	Iodine number	Saturated acids, %	Iso-oleic acid, %	Oleic acid, %	Linoleic acid, %	Iso-linoleic acid, %	Linolenic acid, %
105	115	80 3	23.9	11.2	48.6	1.4	14.1	0.8
99	140	81.9	20.4	16 8	47.3	0.6	14.9	0
110	190	80 1	19.4	23 6	44.7	0.2	12.1	0
85	140	76.1	26.1	13 7	46.0	0	13.9	0.3
102	140	76 6	24.1	15.8	47.0	0.4	12.7	0

Numbers 105, 99, 110 hydrogenated with a commercial catalyst.
Number 85, hydrogenated with oil-reduced nickel formate catalyst.
Number 102, hydrogenated with dry-reduced nickel carbonate catalyst.

not entirely conclusive, evidence for this has been afforded by the following experiments:—

(1) When samples taken throughout a linseed oil hydrogenation were heated under standardized conditions and then scored for odour development, it was found that the linseed oil odour disappeared early in the hydrogenation, but was replaced by the characteristic odour of hydrogenated linseed oil. The intensity of this odour was at a maximum in samples with iodine numbers between 80 and 130; it decreased on further hydrogenation, and was very slight in samples with iodine numbers less than 50. In other words, the intensity of the odour roughly paralleled the accumulation and disappearance of iso-linoleic acid.

(2) Partial hydrogenation of pure methyl linolenate, prepared according to the method of Rollett (14), yielded a product that, when heated, developed an odour similar to that of heated hydrogenated linseed oil, but quite different from the odour of the unhydrogenated ester or from that of pure methyl linoleate, when treated in the same way.

(3) When the distilled iso-linoleic acid concentrate from hydrogenated linseed oil was heated, a very strong odour developed; this was similar to the odour of heated hydrogenated linseed oil.

Discussion

The double bonds in linolenic acid are in the 9 : 10, 12 : 13, and 15 : 16 positions, and in linoleic acid they are in the 9 : 10 and 12 : 13 positions. Van der Veen (16) subjected hydrogenated methyl linolenate to ozonolysis, and concluded from the nature of the breakdown products that hydrogenation caused saturation of the 12 : 13 double bond, yielding a 9 : 10, 15 : 16 methyl linoleate. The results of the spectral absorption measurements on the isomeric linoleic acid from hydrogenated linseed oil support this conclusion,

as double bonds in the 9 : 10 and 15 : 16 positions are too far apart to form a conjugated system on treatment of the oil with alkali.

It has not been definitely established that iso-linoleic acid is the cause of the development of the unpleasant flavour in hydrogenated linseed oil, but there is no doubt that the responsible substance is destroyed by hydrogenation as the difficulty is largely overcome by continuing the hydrogenation until the iodine number is lower than 50; such a product, however, is too hard for use as a shortening. To make an acceptable linseed oil shortening, the substance causing the development of flavour should be hydrogenated completely, but the formation of saturated and iso-oleic acids, which is responsible for the rise in melting point, should be greatly suppressed. To achieve this, a hydrogenation catalyst is required that combines the properties of both exceptionally high selectivity and good iso-oleic acid suppression.

Acknowledgments

References

1. BAILEY, A. E., FEUGE, R. D., and SMITH, B. A.   Oil & Soap, 19 : 169-176.   1942.
2. BAUGHMAN, W. F. and JAMIESON, G. S.   Oil & Fat Industries, 7 : 331-332.   1930.
3. BICKFORD, W. G.   Oil & Soap, 18 : 95-98.   1941.
4. BROWN, J. B. and FRANKEL, J.   J. Am. Chem. Soc. 60 : 54-56.   1938.
5. BROWN, J. B. and STONER, G. G.   J. Am. Chem. Soc. 59 : 3-6.   1937.
6. CARY, H. H. and BECKMAN, A. O.   J. Opt. Soc. Am. 31 : 682-689.   1941.
7. DURKEE, M. M.   Ind. Eng. Chem. 28 : 898-903.   1936.
8. FRANKEL, J. and BROWN, J. B.   J. Am. Chem. Soc. 63 : 1483-1484.   1941.
9. FRANKEL, J. S., STONEBURNER, W. and BROWN, J. B.   J. Am. Chem. Soc. 65 : 259-262.   1943.
10. KAUFMANN, H. P.   Studien auf dem Fettgebiet, Berlin, Verlag Chemie.   1935.
11. MITCHELL, J. H., KRAYBILL, H. R., and ZSCHEILE, F. P.   Ind. Eng. Chem. Anal. Ed. 15 : 1-3.   1943.
12. PAINTER, E. P. and NESBITT, L. L.   Oil & Soap, 20 : 208-211.   1943.
13. RIEMENSCHNEIDER, R. W., SWIFT, C. E. and SANDO, C. E.   Oil & Soap, 18 : 203-206.   1941.
14. ROLLETT, A. Z.   physiol. Chem. 62 : 410.   1909.
15. ROSE, W. G. and JAMIESON, G. S.   Oil & Soap, 18 : 173-174.   1941.
16. VAN DER VEEN, H.   Chem. Umschau Fette, Öle, Wachse Harze, 38 : 89-96.   1931.

FACTORS AFFECTING THE STORAGE OF DEHYDRATED PORK[1]

By Jesse A. Pearce[2]

Abstract

In an accelerated storage test at 60° C. fat levels of 20 and 30% were observed to have no effect on keeping quality of dehydrated pork. Measurements on dried product stored in paper-bodied containers for one year at temperatures from −17.8° C. to 36.7° C. showed 15.6° C. to be less desirable than 23.9°, 0°, or −17.8° C. Dehydrated pork was stored in tin-plate containers for periods of one year at 23.9° C. and 36.7° C. with little decrease in palatability. No difference in storage life was demonstrated between cured and uncured pork, or, as a result of differences in drying times, moisture content or storage temperature. The product prepared on an atmospheric double-drum drier deteriorated a little more rapidly than vacuum–tray- or tunnel–tray-dried material. The effectiveness of container materials, tin-plate, Reynolds' metal *A*-10, Dewey and Almy *P*-16, and 300 *MST* cellophane, was evaluated and their relative value for dehydrated pork fell in that order.

Introduction

A previous paper (2) described methods of dehydrating pork. Second in importance to the satisfactory preparation of dried foods is the determination of factors affecting storage life. This paper records the effect of methods of preparation, storage temperature, moisture content, and types of packages on the keeping quality of dehydrated pork.

Materials

The materials used in this investigation were those prepared in the previous study (2). Tunnel–tray-dried sow ham (uncured) was used in assessing the majority of the factors believed to affect the storage life of dried pork. Comparisons were made between tunnel–tray-, vacuum–tray-, and atmospheric–drum-dried uncured ham, and between uncured and cured tunnel–tray-dried ham.

Analytical Methods

The methods used for determining moisture content, fat content, peroxide oxygen values, and palatability scores (based on a maximum score of 10) have been described (2). The peroxide values are recorded as ml. $0.002\ N$ thiosulphate per gm. of extracted fat.

A preliminary survey had indicated that a fluorescence method may be a useful objective measure of quality of dehydrated pork (3); therefore, this test was used in a portion of the present investigation. The technique used for defatting this product was as follows: the sample was pulverized in a mortar, about 2.5 gm. was defatted by shaking at room temperature with

---

[2] *Biochemist, Food Investigations.*

three 25 ml. portions of petrol ether, the ether was drained off through a No. 1 Whatman filter paper, and the remaining solvent allowed to evaporate. The remainder of the procedure was the same as the revised method for dried whole egg powder (5). The results are recorded in Coleman photo-fluorometer units.

Experimental Methods and Results

The Effect of Fat Content

During preliminary work with dehydrated pork, a product containing about 40% fat was assessed by a taste panel as much less desirable than products with 20 or 30% fat. Therefore in evaluating the effect of fat levels on keeping quality the study was limited to tunnel–tray-dried uncured material with 20 and 30% fat. An accelerated storage trial at 60.0° C. (140° F.) showed that there was little difference in the behaviour of dried pork containing these quantities of fat (Fig. 1). The induction period was slightly reduced
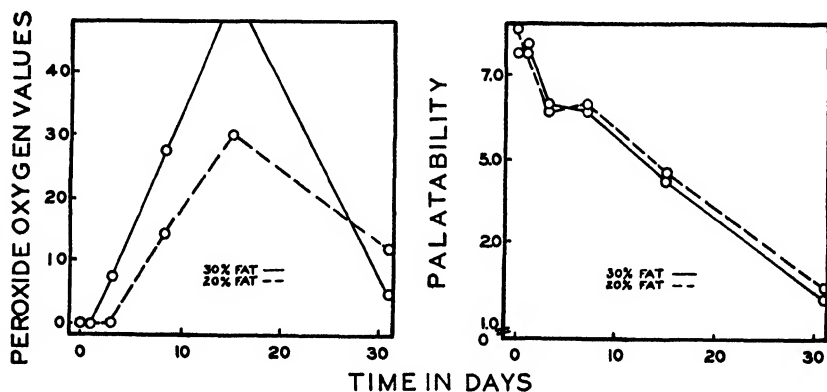
FIG. 1. *The effect of fat content on the quality of dehydrated pork stored at 60.0° C. (140° F.).*

and the maximum value for peroxide oxygen was somewhat greater in the high fat samples. Palatability scores showed that there was a slight preference for the product with the lower fat content: deterioration occurred at about the same rate in both products.

It was concluded that the fat content would have little effect on the rate of deterioration and that studies with material containing 30% fat would be preferable since it is necessary to utilize as much fat as possible and products with this fat content were not distasteful.

The Effect of Storage Conditions

The effect of temperature and humidity was assessed by storing two batches of dehydrated tunnel–tray-dried uncured pork, the one having a moisture content of 6.5% and the other 14%, in paper-bodied containers at −17.8°, 0°, 15.6°, and 36.7° C. (0°, 32°, 60°, and 98° F.) and at relative humidities of 62, 65, 20, and 11% respectively. Several samples were also stored at 23.9° C. (75° F.), relative humidity, 18%. Peroxide oxygen determinations

were made at various times throughout a storage period of one year: palatability was assessed only at the final sampling.

Changes in peroxide value are shown in Fig. 2. At 36.7° C. (98° F.) the peroxide value of both types of dehydrated pork had decreased to zero after
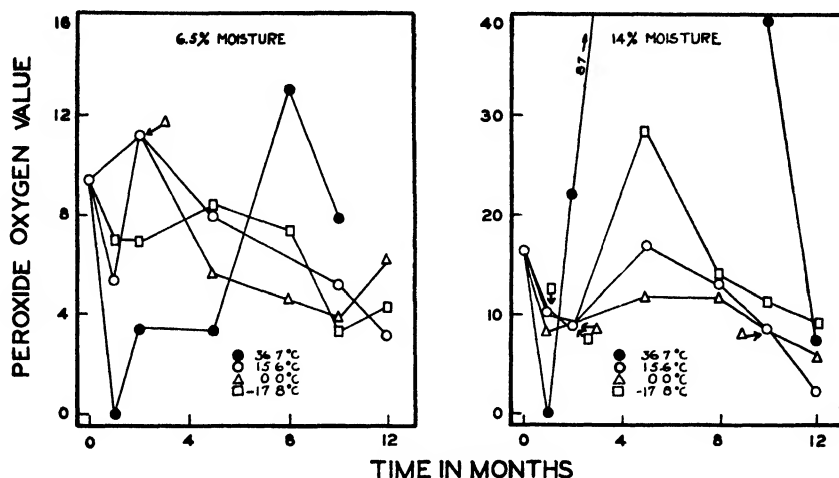
FIG. 2. *Peroxide oxygen changes occurring in dehydrated pork stored at various temperatures in paper-bodied containers.* ● = 36.7° C. (98° F.); ○ = 15.6° C. (60° F.); △ = 0.0° C. (32° F.); □ = −17.8° C. (0° F.).

one month; subsequently the value rose to a maximum at about eight months' storage and then decreased to the end of the storage period. The maximum for the high moisture sample was about seven times as great as that for the low moisture sample. At the lower temperatures the trends of the curves are generally similar; a slight decrease during the early part of the storage is evident, followed by an increase and finally a gradual sloping off.

On the basis of palatability measurements (Table I), the samples receiving the lowest scores were those stored at 15.6° C. (60° F.) and 36.7° C. (98° F.), the scores for product at both moisture levels being practically the same. The lower score at the higher temperature was to be expected; however,

TABLE I

PALATABILITY SCORES FOR DEHYDRATED PORK WITH 6.5 AND 14% MOISTURE AFTER STORAGE
FOR 12 MONTHS IN PAPER-BODIED CONTAINERS (INITIAL PALATABILITY, 6 6)

Storage temperature		Palatability score	
°C.	°F.	6.5% Moisture	14% Moisture
36.7	98	3.0	2.7
23.9	75	4.1	
15.6	60	3.2	2.9
0	32	4.2	3.9
−17.8	0	5.5	5.2

explanation of the low scores at 15.6° C. (60° F.) would be highly speculative. It may be that this was an optimum temperature for enzyme activity, a type of deterioration possibly differing from that occurring at the higher temperatures. Similar behaviour was noted during the storage of dehydrated mutton (6).

Since the relative humidities in the storage cabinets varied appreciably, it was believed desirable to determine the moisture content of some of the stored samples. With the exception of samples stored below the freezing point, the moisture content was related to the relative humidity (Table II).

TABLE II

CHANGES IN MOISTURE CONTENT OF DEHYDRATED PORK STORED AT VARIOUS TEMPERATURES
IN PAPER-BODIED CONTAINERS

Initial moisture, %	Temperature		Relative humidity, %	Moisture content (%) after storage for:	
	°C.	°F.		Two months	Five months
6.5	36.7	98	11	3.6	3.2
	15.6	60	20	5.0	5.4
	0	32	65	8.0	8.9
	−17.8	0	62	8.8	13
14	36.7	98	11	3.6	3.9
	15.6	60	20	5.3	5.9
	0	32	65	9.2	9.4
	−17.8	0	62	13	13

It is evident from these results that, while low temperature storage of dehydrated pork was preferable, the product stored at 23.9° C. (75° F.) was as edible as the product stored for the same period at 0° C. (32° F.). As a result, investigation of other factors was continued only at the higher temperatures.

The Effect of Curing

It has been pointed out that cured ham suffered slightly greater deterioration during the tunnel–tray-drying process, although dried cured ham when reconstituted was usually rated higher than the product from uncured material (2). Peroxide values for dried cured ham and uncured ham stored for one year in tin-plate containers at 23.9° C. (75° F.) and 36.7° C. (98° F.) are given in Fig. 3, A.

The changes in peroxide values were similar for both types of dried product. The samples stored at the lower temperature reached a maximum value after one month's storage, then slowly decreased. Those at the higher temperature decreased rapidly to zero.

Mean palatability scores are given in Table III: none of the factors studied were significant. Since cured ham is more costly, nothing would be gained by using it as the initial material.
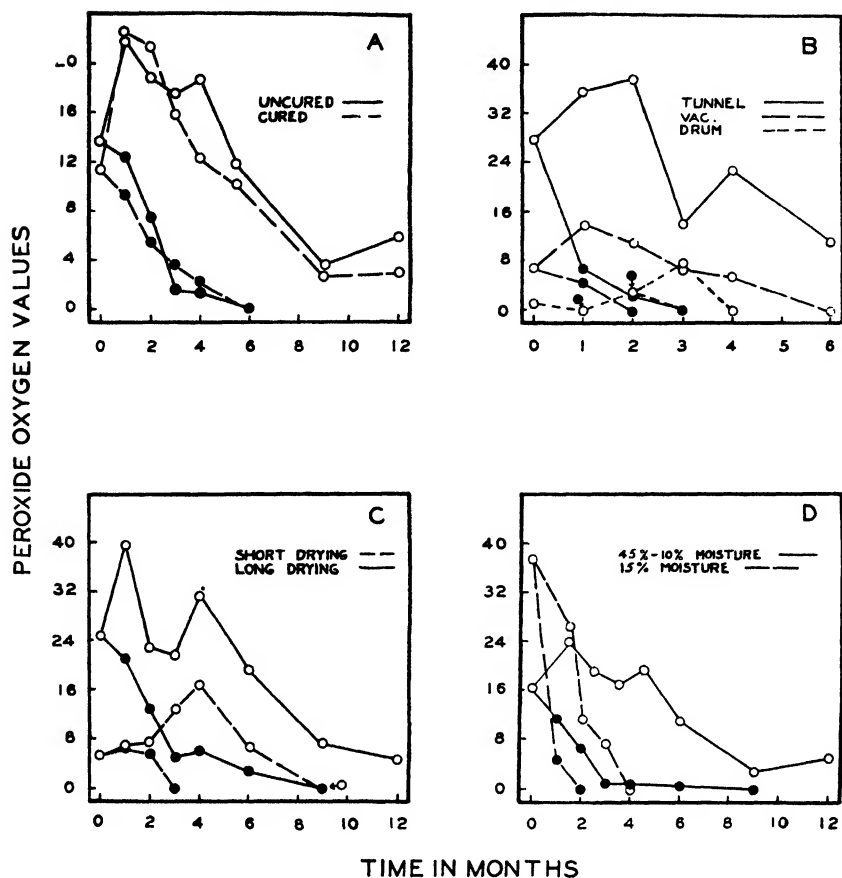
FIG. 3. *Effect of curing (A), method of drying (B), time of drying (C), and moisture content (D) on peroxide oxygen changes in the fat of dehydrated pork.* ● = 36.7° C. (98° F.); O = 23.9° C. (75° F.).

Effect of Method of Drying

Three drying techniques were investigated during the course of earlier work (2). While tunnel–tray drying produced the best initial product, it was nevertheless believed desirable to measure the keeping quality of uncured pork dehydrated by each of the three methods. The products prepared by different methods of drying had peroxide values increasing in magnitude as follows: atmospheric double-drum, vacuum–tray, and tunnel–tray. It was interesting to note (Fig. 3, B) that the peroxide values again increased to maximum in samples stored at 23.9° C. (75° F.) and then gradually decreased; the maxima were higher for the dried product with greater initial values. At 36.7° C. (98° F.) the peroxide values decreased rapidly to zero.

The significant differences between methods of drying shown in Table IV. could for the most part be explained by differences in initial quality; however, pork dried on the double drums deteriorated a little more rapidly during the

TABLE III

MEAN PALATABILITY SCORES FOR TUNNEL–TRAY-DRIED, CURED AND
UNCURED PORK, STORED IN TIN-PLATE CONTAINERS FOR ONE YEAR
AT 36.7° C. (98° F.) AND 23.9° C. (75° F.)

Variable under investigation	Mean palatability score
Type of material	
Tunnel–tray-dried, cured ham	7.6
Tunnel–tray-dried, uncured ham	7.3
Storage temperature	
36.7° C. (98° F.)	7.4
23.9° C. (75° F.)	7.5
Storage time (months)	
Initial	7.5
1	7.3
2	7.5
3	7.6
4	7.7
6	7.8
9	7.2
12	7.0

last half of the storage period. Therefore the tunnel–tray method, which produces the best initial material, was preferred.

Effect of Drying Period

In the previous experiments it was observed that the dried product with a higher initial peroxide value attained a greater maximum value after a short storage period: this higher initial value was generally indicative of excessive time in the drier. It seemed desirable then to compare the storage life of samples with approximately equal moisture contents but dried for different times and consequently possessing different initial peroxide values.

Batches of about the same moisture content prepared from uncured pork, tunnel–tray-dried under the same conditions for different times, were stored at 23.9° C. (75° F.) and 36.7° C. (98° F.) in tin-plate containers. One batch had a moisture content of 4.6% and an initial peroxide value of 5.4 while the other had 4.8% moisture and a peroxide value of 25.

At the lower temperature, the peroxide oxygen values reached a maximum after a short period in storage, and again the maximum was greater for the product with the greater initial value (Fig. 3, C). At the higher temperature the values decreased gradually to zero, the time required for the decrease being somewhat longer for samples with the higher initial value. Palatability was generally lower for the product with the high peroxide values (Table V), changes during storage being comparable in each case. Again temperature and time were without significant effect. It could be assumed that, except for a general lowering of the palatability values, commencing a storage experiment with dried pork having a high initial peroxide value would not materially alter the rate of decrease of palatability.

TABLE IV

MEAN PALATABILITY SCORES AND ANALYSIS OF VARIANCE FOR PORK
DRIED BY DIFFERENT METHODS, STORED IN TIN-PLATE CONTAINERS
FOR ONE YEAR AT 36.7° C. (98° F.) AND 23.9° C. (75° F.)

A. Table of means

Variable under study	Mean palatability score
Method of drying	
Tunnel–tray	7.7
Vacuum–tray	7.2
Atmospheric double-drum	6.6
Necessary difference, 5% level	0.53
Storage temperature	
36.7° C. (98° F.)	7.1
23.9° C. (75° F.)	7.2
Storage time (months)	
Initial	6.5
1	7.2
2	7.5
3	7.1
4	7.2
6	6.9
Necessary difference, 5% level	0.37

B. Analysis of variance

Variance attributable to:	D.f.	Mean square
Method of drying	2	2.87**
Temperature	1	0.05
Time	4	0.32*
Method × time	8	0.40**
Residual (error)	13	0.05

* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% level of statistical significance.*

Effect of Moisture Content

In the experiment on storage temperatures the samples employed had different initial moisture contents and these were observed to change during storage. Since low moisture content retards deterioration in stored dried egg powder (7), the storage behaviour of these dried pork samples might likewise be attributed to moisture effects. Therefore, tunnel–tray-dried uncured pork with a range of moisture contents (4.5 to 15%) was stored in tin-plate containers at 23.9° C. (75° F.) and 36.7° C. (98° F.).

The results in Fig. 3, D revealed no difference between the average peroxide values for products with moisture contents from 4.5 to 10%; the behaviour

TABLE V

MEAN PALATABILITY SCORES AND ANALYSIS OF VARIANCE FOR PORK
TUNNEL–TRAY-DRIED FOR DIFFERENT PERIODS STORED IN TIN-PLATE
CONTAINERS FOR ONE YEAR AT 36.7° C. (98° F.) AND
23.9° C. (75° F.)

A. Table of means

Variable under study	Mean palatability score
Dehydration period	
Short	7.8
Long	7.0
Storage temperature	
36.7° C. (98° F.)	7.3
23.9° C. (75° F.)	7.5
Storage time (months)	
Initial	7.2
1	7.6
2	7.8
3	7.1
4	7.6
6	7.8
9	7.0
12	7.0

B. Analysis of variance

Variance attributable to:	D.f.	Mean square
Dehydration period	1	3.94*
Temperature	1	0.13
Time	6	0.59
Residual (error)	18	0.62

* *Exceeds 5% level of statistical significance.*

was similar to that previously described. The samples with 15% moisture had a higher initial peroxide value and are shown separately. This product, when stored at 23.9° C. (75° F.) behaved unlike any of the samples previously studied; the values decreased to zero without passing through a maximum. This decrease was somewhat slower than for the comparable material at the higher temperature.

On the basis of palatability scores, it appeared from the results in Table VI that temperature was without effect, but both moisture and time appeared to have significant effects. However the apparent effect of moisture was due to difference in initial quality. It was concluded that a low moisture content did not extend the storage life of dehydrated pork.

It should be noted here that the samples used in all experiments up to this point were either quickly and carefully packed on removal from the drier, or

TABLE VI

MEAN PALATABILITY SCORES AND ANALYSIS OF VARIANCE FOR
DEHYDRATED PORK WITH DIFFERENT MOISTURE CONTENTS STORED
IN TIN-PLATE CONTAINERS FOR ONE YEAR AT 36.7° C. (98° F.)
AND 23.9° C. (75° F.)

A. Table of means

Variable under study	Mean palatability score
Moisture content (%)	
4.5	7.9
5.0	7.0
5.5	7.5
6.0	7.5
6.5	7.1
10.0	7.5
15.0	7.4
Necessary difference, 5% level	0.37
Storage temperature	
36.7° C. (98° F.)	7.4
23.9° C. (75° F.)	7.4
Storage time (months)	
Initial	7.1
1	7.6
2	7.6
3	7.2
4	7.6
6	7.7
9	7.1
12	6.9
Necessary difference, 5% level	0.37

B. Analysis of variance

Variance attributable to:	D.f.	Mean square
Moisture content	6	0.91**
Temperature	1	0
Time	6	1.26**
Residual (error)	78	0.12

** *Exceeds 1% level of statistical significance.*

held in sealed containers at 4.4° C. (40° F.) until packed. A number of samples with different moisture contents were kept on the laboratory shelves in quart sealers. These were opened occasionally for demonstration purposes. Mould growth sufficient to render the dried product inedible appeared within one month on those samples with moisture contents greater than 13%, in two months the sample with 13% moisture was also mouldy, while for moisture contents of 10% or less no moulds appeared after nine months. This indicated that moisture contents of less than 10% were desirable.

Effect of Packaging Materials

Comparison of the palatability scores in Tables I and III indicated that tin-plate was preferable to paper-bodied containers. Direct comparison of methods of packaging was therefore made on tunnel–tray-dried uncured ham with a fat content of 32% and a moisture content of 4.0%. The material was stored at 26.7° C. (80° F.), at 16 and 85% relative humidity, and at 37.8° C. (100° F.) and 11% relative humidity in tin-plate (uncompressed) and as compressed blocks in three types of substitute containers: 300 *M.S.T.* cellophane in cardboard; 300 *M.S.T.* cellophane in cardboard, finished container wax dipped (Dewey and Almy *P*-16); 300 *M.S.T.* cellophane in cardboard with an overwrap of Reynolds' metal *A*-10. 'The finished package contained about 300 gm. of meat and measured 1¾ in. × 3½ in. × 4 in.

Measurements of moisture gain were made for a four-week period on packages stored at the high humidity. Comparison was made of packages carefully handled and of packages dropped six times from a height of three feet. (The latter packages were used for the quality tests since it was considered that the treatment that they had received would approximate handling conditions during transport.) Table VII shows the mean values for three packages of each type measured at weekly intervals, omitting the gains during the first week since these are complicated by increases resulting from sorption of moisture on the packaging materials. These values indicated

TABLE VII

Mean weekly moisture gain (gm.) of dehydrated pork in packages with various moisture barriers stored at 26.7° C. (80° F.) and 85% relative humidity

Moisture barrier	Method of handling	
	With care	After dropping
Tin-plate	0	0
Reynolds' Metal *A*-10	0.19	0.25
Dewey and Almy *P*-16	0.21	1.19
300 *MST* cellophane	1.56	2.27

that Reynolds' metal and wax-dipping were about equally effective if carefully handled, while Reynolds' metal was the better barrier after rough treatment. The Reynolds' metal used here behaved differently from that used in another experiment (4), in that it showed no evidence of breaking down under the high humidity conditions.

The results of palatability and fluorescence measurements are shown in Table VIII; peroxide oxygen changes were again of little value (Fig. 4) although it might be noted here that the initial value was zero. The fluorescence changes indicated that storage at low temperature and low humidity was preferable while the palatability scores showed significant deterioration

TABLE VIII

MEAN PALATABILITY SCORES AND FLUORESCENCE VALUES FOR DEHYDRATED PORK STORED IN
CONTAINERS WITH DIFFERENT MOISTURE BARRIERS FOR ONE YEAR UNDER
VARIOUS CONDITIONS

A. Table of means

Variable under study	Mean palatability score	Mean fluorescence value
Moisture barriers		
Tin-plate	7.7	48.0
Reynolds' metal *A*-10	7.1	47.4
Dewey and Almy *P*-16	6.4	54.0
300 *MST* cellophane	6.1	50.2
Storage condition		
37.8° C. (100° F.) 11% R.H.	7.0	60.2
26.7° C. (80° F.) 85% R.H.	6.1	49.8
26.7° C. (80° F.) 16% R.H.	7.4	39.6
Necessary difference, 5% level	—	11.2
Storage time		
Initial	8.5	20.2
3	6.8	47.9
6	7.8	48.5
12	5.8	53.3
Necessary difference, 5% level	1.6	—

B. Analysis of variance

Variance attributable to:	Palatability scores		Fluorescence values	
	D.f.	Mean square	D.f.	Mean square
Moisture barriers	3	4.61	3	81
Storage condition	2	5.51	2	1275**
Storage time	2	12.32*	2	104
Residual (error)	28	2.33	26	90

* Exceeds 5% level of significance.
** Exceeds 1% level of significance.

after one year of storage. Neither criterion showed significant differences in package types. This failure to show a difference could perhaps be attributed to the fact that the mouldy portions of the samples were removed prior to testing.

Mould growth on samples stored at 26.7° C. (80° F.) and 85% relative humidity showed the relative effectiveness of the packaging materials. Samples in tin-plate did not become mouldy, in Reynolds' metal *A*-10 they were slightly mouldy after one year's storage, and in the wax-dipped cartons they were quite mouldy; samples in cellophane only were slightly mouldy after six months'

storage and inedible at the final sampling. Since samples of high moisture content when handled carefully showed no evidence of mould growth, it is likely that these samples were infected during compression.
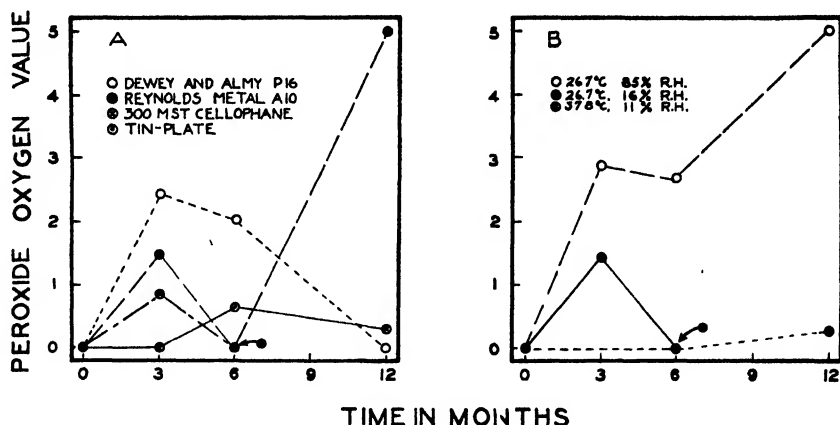
FIG. 4. *Effect of method of packaging (average for all temperatures) and of temperature (average for all methods of packaging) on the peroxide oxygen behaviour of dehydrated pork fat.*

Discussion

Throughout the study it was noted that there was generally an increase in palatability of dehydrated pork after it had been stored for a short period. This increase in palatability coincided with an increase in strong odour evident when the containers were opened: the odour disappeared after a few minutes' standing. Therefore, this initial increase in palatability was believed to result from the release during storage of volatile breakdown products formed during the drying process.

The fat fraction of fresh pork in chill or freezer storage is a relatively unstable material and the development of peroxide oxygen is an excellent indication of organoleptically detectable rancidity (1). The dried product differs markedly in behaviour; the fatty fraction of the material may show very high peroxide oxygen values without any rancidity detectable by taste panels. Explanation of this behaviour is difficult. It might be suggested that the formation of peroxide in fresh pork fat occurs at about the same rate as the deterioration actually causing rancidity in the fat, but that this deterioration is prevented as a result of the destruction of enzymes, or the formation of inhibitors during the drying process. During the storage of dried pork only the oxidative change would be taking place, hence, high peroxide oxygen values without organoleptic rancidity.

Calculations on the basis of moisture gain indicate that the product packed in Reynolds' metal should have attained a moisture content of about 8% only after one year's storage. Since moulds grew only on samples with more than 13% moisture, the presence of mould growth on the final sample must then be the result of uneven distribution of moisture through the compressed

block. The outer surfaces, where mould growth occurred, would exceed the desirable moisture limit. This feature must be considered when writing specifications for dehydrated pork to be packed in substitute containers, and to be stored for long periods at high humidity.

Acknowledgments

References

1. Cook, W. H. and White, W. H.   Can. J. Research, D, 19 : 53-60.   1941.
2. Pearce, J. A.   Can. J. Research, D, 21 : 394-404.   1943.
3. Pearce, J. A.   Can. J. Research, F, 22 : 87-95.   1944.
4. Pearce, J. A. and Marshall, J. B.   Can. J. Research, F, 23: 22-38.   1945.
5. Pearce, J. A., Thistle, M. W., and Reid, M.   Can. J. Research, D, 21 : 341-347.   1943.
6. Vickery, J. R.   Private communication.
7. White, W. H., and Thistle, M. W.   Can. J. Research, D, 21 : 211-222.   1943.

RATION BISCUITS

I. FACTORS AFFECTING THE KEEPING QUALITY OF BISCUITS CONTAINING PROTEIN SUPPLEMENTS

By Jesse A. Pearce and J. B. Marshall

RATION BISCUITS

I. FACTORS AFFECTING THE KEEPING QUALITY OF BISCUITS CONTAINING PROTEIN SUPPLEMENTS[1]

By Jesse A. Pearce[2] and J. B. Marshall[3]

Abstract

The storage life of biscuits was extended by maintaining a moisture content of 6% or less.   From considerations of initial quality and of increased storage life sodium bicarbonate was superior to ammonium bicarbonate as a leavening agent, particularly at lower levels.   By the same criteria, wheat germ appeared to be a more satisfactory source of protein than soy flour, dried skim milk, or dried egg yolk.

Moisture-resistant packaging materials were found necessary to prevent mould growth and maintain edibility in biscuits stored at high humidities; however, biscuits so packed deteriorated at a faster rate at high temperatures. Tin-plate containers appeared to be the most effective for long term storage at high humidities, although Reynolds' metal A-10 with or without an inner liner was satisfactory for storage periods of about half a year.

While none of the quality measurements used were completely reliable measures of biscuit quality, a fluorescence measurement assessed the effects of the various treatments in a relatively satisfactory manner.

Introduction

Biscuits were among the earliest forms of dehydrated food.   They have been widely used for victualing ships, and as reserve rations for troops and expeditions that may be separated from ordinary sources of supply for protracted periods.   The old style ships' biscuit and hard tack were made from flour, salt, soda, and a very small amount of shortening.   They were very hard, dry, and difficult to masticate.

Various types of biscuits have been used extensively in normal diets.   Among these are zwei-back, soda crackers, and various sorts of 'health biscuit'. Improvements in the eating quality and nutritional properties of these materials have been made by increasing the fat and protein content of the formula and by adding sugar and other ingredients to enhance the flavour.   However these additions have complicated the factors affecting the keeping qualities of the product and raised questions that can be answered only by comprehensive storage experiments.

The keeping quality of foodstuffs is affected by many factors.   The type of package is obviously very important.   Moisture content has been shown to be an important factor in keeping quality of dried whole egg powder (14) and wheat germ (3).   Sodium bicarbonate was found to affect the keeping quality

of dried whole egg powder (7). It has also been pointed out that the use of ammonium bicarbonate in place of sodium bicarbonate as a leavening agent resulted in greater conservation of thiamin during baking (2). Various proteinagenous materials such as milk powder, wheat germ, soy flour, and dried egg yolk exhibit different keeping qualities, which in turn might be expected to influence the quality of the biscuits.

The overall change taking place during the spoilage of food material may be due to deterioration of one or more of the component fractions, i.e., fat, protein, or carbohydrate, and may be assessed by organoleptic tests without differentiating between the possible causes of the offensive taste or odour. Thus the term 'rancid' is frequently used loosely. to describe taste reaction to any off-flavour although it should be reserved to describe the condition of spoiled fat. Subjecting tasters repeatedly to large numbers of samples is disturbing and generally results in a less careful judgment towards the end of a long study. A test designed to follow changes in fats, e.g. peroxide oxygen measurements, may fail completely when applied to a composite material in which spoilage of the other components may precede deterioration of the fat (11). Therefore some other objective tests have been used to assess deterioration in this foodstuff and attempts to relate these to organoleptic data have met with varying success.

Many of the factors relating to storage of foodstuffs discussed above are being investigated in these laboratories. This paper deals with an investigation of the effects of temperature, relative humidity, package materials, amount and kind of leavening agent, and type of protein, on the stability of biscuits during storage.

Materials

The standard mix for biscuits used in this experiment was: soft wheat flour, 90 lb.; whole wheat flour, 10 lb.; shortening, 12 lb.; sugar, 5 lb.; dry, powdered, skim milk, 8 lb.; salt, $1\frac{1}{2}$ lb.; and sodium bicarbonate, 12 oz. The moisture content of the finished product was not to exceed 6%. All quantities and variations discussed will be in terms of this recipe.

To obtain an estimation of the effect of method of packaging, biscuits of standard mix were stored at temperatures of 26.7° (16% and 85% relative humidities), 43.3°, and 60.0° C. (80°, 110°, and 140° F.) in 300 *MST* cellophane, cellophane in tin-plate containers, tin-plate without cellophane, in Reynolds' metal *A*-10, and in Reynolds' metal plus cellophane.

For the first experiment with varying moisture content, moisture levels were adjusted, on biscuits prepared from standard mix, to 5.9, 7.1, and 10.3%, by the method described for dried whole egg powder (14). These biscuits were packaged in Reynolds' metal *A*-10 and stored at 26.7° and 43.3° C. (80° and 100° F.).

In the second experiment biscuits made from a mix using 4 oz. sodium bicarbonate and from a mix using 4 oz. ammonium carbonate were dried *in vacuo* over calcium chloride and stored at 26.7° and 43.3° C. (80° and 110° F.)

in tin-plate containers with sufficient water to bring the moisture contents to average values of 3.1%, 4.8%, 6.3%, 7.8%, and 9.1%. These levels were attained during the first week of storage.

To investigate leavening agents the standard mix was varied to include the following levels of sodium bicarbonate; 4 oz., 10 oz., 16 oz., and 22 oz.; and also 4 oz. ammonium bicarbonate and a mixture of 2 oz. ammonium bicarbonate and 2 oz. sodium bicarbonate. The finished biscuits were stored at 43.3° C. (110° F.) in Reynolds' metal A-10.

TABLE I

COMPOSITION OF RATION BISCUITS

Type of biscuit	Composition, %			
	Protein	Starch	Fat	Moisture
Dried milk	9.3	60.0	9.3	7.6
Wheat germ	9.3	61.6	10.6	5.4
Dried egg yolk	9.1	61.4	10.6	4.7
Soy flour	9.5	62.2	8.9	5.6

Replacing dried skim milk powder by various protein components necessitated some variation in the standard mix. These were necessary to maintain a constant ratio of protein, starch, and fat. In the first substitution, the milk powder was replaced by 6 lb., 14 oz. of defatted wheat germ; in the second, 10 lb. of powdered egg yolk replaced the skim milk and the amount of shortening was reduced from 12 lb. to 8 lb., 6 oz. The third substitution for milk powder was 7 lb., 2 oz. of debittered full fat soya flour with a reduction of shortening from 12 lb. to 10 lb., 6 oz. The protein, starch, fat, and moisture contents of the resulting biscuits are shown in Table I. These biscuits were stored at 26.7° (16% and 85% relative humidities), 43.3°, and 60.0° C. (80°, 110°, and 140° F.) in 300 *MST* cellophane, in Reynolds' metal A-10, and in Reynolds' metal plus cellophane.

Methods

Since the ultimate criterion of quality must be edibility of the product, palatability measurements were made by panels of 14 people, each person sampling the biscuits and commenting as follows: excellent, very good, good, fairly good, fair, fair to poor, poor, very poor, and inedible. For ease in averaging the final data, these descriptive categories were assigned numerical values of 8 to 0, respectively.

A fluorescence measurement had proved useful as a test of quality in dried whole egg powders (6, 10). It appeared to measure breakdown of the proteins present in dried egg powders (4), and fluorescence changes have been observed in foods of high carbohydrate content (5). In addition, all the protein com-

ponents selected for use in this study were known to have a fluorescence that varied more or less with storage conditions (3, 4, 5); therefore, this measurement seemed appropriate for the present study. Fluorescence measurements were made in a manner similar to the revised method used for dried whole egg powder (6); the results are recorded in units of the Coleman photofluorometer.

Changes in acidity of stored flour and wheat germ are known to occur (1, 9). Work on dried egg powders indicated that the pH of potassium chloride extracts of defatted powder was related to storage treatment (14). Since it has been noted that alcohol extracts contained a portion of the fluorescing substances, the amount varying in quantity with the quality of the egg powder (4), it was believed that pH measurements on alcohol extracts of biscuits might prove to be a good measure of quality. Therefore pH measurements were made on alcohol (95%) extracts of the whole biscuits after dilution of the alcohol extract to 50%, on extracts of defatted biscuits in 10% sodium chloride, and on extracts of whole biscuits in 10% potassium chloride. This last type of extract should reflect changes in acidity occurring in the whole biscuit, the second, changes in the non-fatty portion, and the first, changes in the fatty portion although some amino acids and protein matter might be present (8). The average pH of the various solvents was as follows: 10% potassium chloride, 5.2; 10% sodium chloride, 5.5; and alcohol, when diluted, 6.2.

To reduce interference resulting from deterioration of the fat, the most stable shortening obtainable was used (13). Nevertheless, it was felt desirable to measure peroxide oxygen development in the fat fraction of the biscuits. These measurements were made on fat extracted from the biscuits; the technique of this determination has been described (12). Development of peroxide oxygen values was slow, a month's storage at 60.0° C. (140° F.) and eight months' storage at 26.7° C. (80° F.) being required to bring the value to about 1 ml. 0.002 N thiosulphate per gram of extracted fat. The biscuits had deteriorated to a marked extent for other reasons and hence peroxide oxygen values were of little importance and are not discussed.

The withdrawal of packages at the various time periods was done by a randomized procedure. Sampling the contents of the selected packages for palatability scores and objective tests differed somewhat. Four biscuits from a package were broken into four pieces each and one piece sampled by a taster, while for the objective measurements one analysis was done on each of two biscuits from the same package. This method was used because the error of the quality measurements, appreciable between biscuits, was thought to be greater than between packages. Furthermore, it was known that the analytical error was much smaller than the between-biscuit error.

Results

Effect of Packaging

The data obtained with five different types of packages are shown in Fig. 1. At 60·0° C. (140° F.) fluorescence development in biscuits stored for four weeks differed for each type of package. The material packaged in cellophane
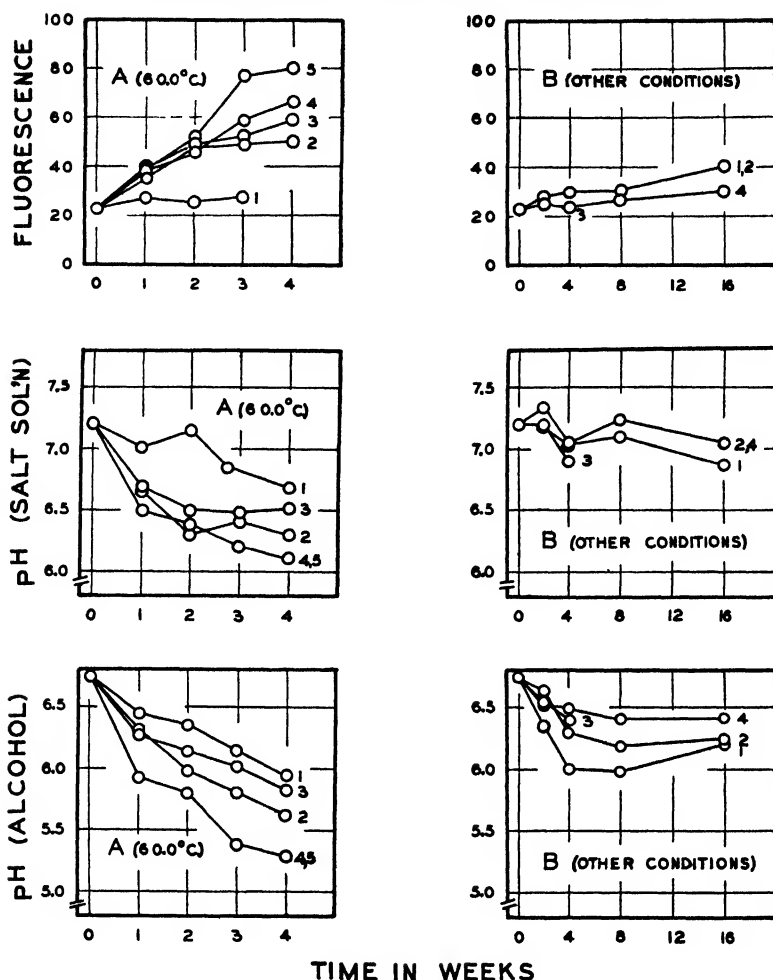
FIG. 1. *Changes in fluorescence values and pH of extracts of biscuits stored in different packaging materials.*

A, at 60.0° C. (140° F.): Curve 1, 300 MST cellophane, Package (a); Curve 2, in Reynolds' metal A-10, Package (b); Curve 3, in 300 MST cellophane and Reynolds' metal A-10, Package (c); Curve 4, in tin-plate, Package (d); Curve 5, in 300 MST cellophane and tin-plate, Package (e).

B, at other conditions: Curve 1, Packages (b), (c), (d), and (e), at 43.3° C. (110° F.); Curve 2, Package (a) at 26.7° C. (80° F.) and 85% relative humidity; Curve 4, all other packages at 26.7° C. (80° F.).

alone developed fluorescing substances more slowly than any of the others. Similar changes occurred in biscuits packed in tin-plate and in Reynolds' metal, except that slightly higher values developed in the biscuits packaged in the former: the additional cellophane wrapping seemed to accentuate changes occurring in biscuits stored in Reynolds' metal or in tin-plate.

Fluorescence values showed no difference in biscuits packaged in various materials and stored for 16 wk. at 43.3° C. (110° F.) and at 26.7° C. (80° F.)

and 16% relative humidity although fluorescence development was slightly greater at the higher temperature. Biscuits packaged in cellophane and stored for four weeks at 26.7° C. (80° F.) and 85% relative humidity developed fluorescence values at the same rate as biscuits in other packages, but at six weeks they showed some mould growth and at eight weeks were too mouldy for measurements. Biscuits in the other types of packages behaved in the same manner as those stored at the lower humidity.

Increases in acidity were noted in biscuits stored at all conditions, the greatest changes occurring in those packaged in tin-plate and stored at 60° C. (140° F.). The least change occurred in the material wrapped in cellophane only. At 43.3° C. (110° F.) cellophane wrapped biscuits developed acid products more slowly than biscuits packed in other materials. Biscuits packaged in cellophane and stored at 26.7° C. (80° F.) and 85% relative humidity decreased in pH more rapidly than biscuits in all other types of package stored at both humidities. Changes in pH of the alcohol extracts of these biscuits showed the same trends as that of the sodium chloride extracts, although the general level was lower.

On the basis of the foregoing measurements there seemed to be little difference in the behaviour of materials packaged in Reynolds' metal and tin-plate, except at high temperatures, where it appeared that tin-plate had a more drastic effect on the contents than the substitute packaging materials. Most surprising was the adverse effect resulting from the addition of a cellophane wrap on materials stored at the high temperature, indicating that under this condition materials with higher moisture vapour transmission extended storage life. As a result of this experiment, cellophane packages seemed to merit further study. Reynolds' metal was selected also for use in further studies, since any changes occurring in tin-plate could be expected to be even more drastic at high temperatures and similar at low temperatures.

Effect of Moisture

Table II, showing mean values, indicates the effect of moisture levels on biscuits of standard mix as assessed by fluorescence values and pH measurements on the sodium chloride extract of the defatted biscuits. These results show the effect of moisture content in increasing the development of fluorescing substances. An increase in moisture content from 6 to 7% caused a significant increase in fluorescing substances. A further increase to 10% had a less marked effect on the rate of fluorescence development. Since the range of conditions was limited, pH measurements did not show a significant effect due to moisture content although the trends indicated by the mean values support observations on the basis of fluorescence development.

It was concluded from this experiment that an investigation of a still greater range of moisture levels was necessary. It was believed expedient to include a study of the effect of different leavening agents. The results of this second experiment are given in Tables III and IV.

TABLE II

EFFECT OF MOISTURE ON KEEPING QUALITY OF BISCUITS MADE FROM THE STANDARD MIX AND
STORED 15 WK. AT 43.3° C. (110° F.)

A. Table of means

Variable under study	Fluorescence (photofluorometer units)	pH of sodium chloride extract
Time, wk. Initial	27.1	7.92
1	27.4	7.44
2	29.7	7.09
6	32.5	7.16
15	33.1	6.85
Necessary difference, 5% level	1.68	0.20
Moisture content, % 5.9	27.5	7.38
7.1	30.4	7.25
10.3	32.0	7.25
Necessary difference, 5% level	1.30	—
Temperature 26.7° C. (80° F.)	28.1	7.34
43.3° C. (110° F.)	59.9	7.25

B. Analysis of variance

Variance attributable to:	D.f.	Mean square Fluorescence (photofluorometer units)	pH of sodium chloride extract
Temperature	1	102.67**	0.0599
Moisture	2	51.26**	0.0517
Time	4	46.48**	1.0228**
Temperature × moisture	2	10.31*	0.0909
Time × temperature	4	40.60**	0.0295
Moisture × time	8	6.32*	0.0332
Temperature × moisture × time	8	1.60	0.0225

* Exceeds 5% level of statistical significance.
** Exceeds 1% level of statistical significance.

TABLE III

PALATABILITY SCORES OF STORED BISCUITS AT DIFFERENT MOISTURE LEVELS,
AVERAGE FOR TWO TYPES OF LEAVENING AGENT

Moisture content, %	Palatability scores 43.3° C. (110° F.) 11 wk.	26.7° C. (80° F.) 15 wk.
3.1	3.7	5.4
4.8	3.9	5.4
6.3	3.6	5.2
7.8	2.7	4.4
9.1	2.8	4.2
Necessary difference, 5% level	0.7	0.7

TABLE IV

EFFECT OF MOISTURE ON KEEPING QUALITY OF BISCUITS CONTAINING DIFFERENT LEAVENING AGENTS AND STORED 11 WK. AT 43.3° C. (110° F.)

A. Table of means

Variable under study	Fluorescence (photofluorometer units)		pH of potassium chloride extract	
	Biscuit 1	Biscuit 2	Biscuit 1	Biscuit 2
Time, wk. Initial	19.2	28.7	6.60	6.60
1	20.6	30.6	6.60	6.69
3	19.3	31.4	6.58	6.68
7	16.4	24.9	6.47	6.50
11	21.1	34.6	6.47	6.48
Necessary difference, 5% level	2.9	4.7	0.04	0.02
Moisture content, %				
3.1	17.9	36.8	6.55	6.63
4.8	17.7	27.2	6.56	6.64
6.3	18.7	32.8	6.52	6.62
7.8	19.8	38.2	6.51	6.57
9.1	22.7	38.2	6.43	6.47
Necessary difference, 5% level	3.4	5.3	0.05	0.03

B. Analysis of variance

Variance attributable to:	D.f.	Mean square			
		Fluorescence (photofluorometer units)		pH of potassium chloride extract	
		Biscuit 1	Biscuit 2	Biscuit 1	Biscuit 2
Time	3	44.01**	164.85**	0.0845**	0.1245**
Moisture content	4	33.35**	106.45**	0.0186**	0.0409**
Moisture content × time	12	7.99	24.98	0.0048**	0.0837**
Between biscuits	20	4.74	12.80	0.0011	0.0003

**Exceeds 1% level of statistical significance.

Table III shows the mean palatability scores (mean scores by eight tasters) combined for both types of biscuits (4 oz. sodium bicarbonate compared with 4 oz. ammonium bicarbonate as leavening agent) after storage. Statistical analyses showed that the types of biscuits did not differ, the only significant effects being those due to tasters, to moisture contents, and temperature. It is evident from this table that the biscuits between 3.1 and 6.3% moisture did not differ after storage but were of higher quality than biscuits at 7.8 and 9.1% moisture.

The quality measurements used, i.e. fluorescence value, pH of potassium chloride, sodium chloride, and alcohol extracts were generally not satisfactory.

The only significant changes were in fluorescence substances and in pH of the potassium chloride extracts of biscuits stored at 43.3° C. (110° F.). Only the significant results are given in Table IV; these show considerable variation in the values obtained by the chemical analyses, but tend to support the conclusions drawn from palatability scores.

The above results indicate that the storage life of biscuits was extended by maintaining moisture content in the biscuits of 6% or less.

Effect of Soda Level

The results of objective tests on biscuits containing varying amounts of sodium bicarbonate, ammonium bicarbonate, and both sodium and ammonium bicarbonate are shown in Fig. 2.
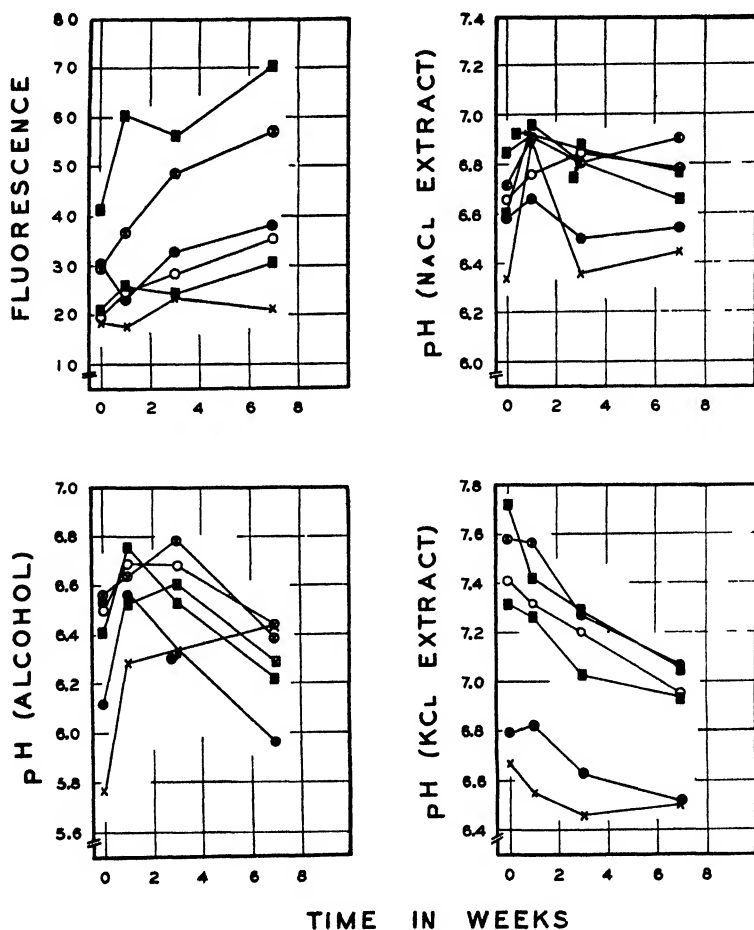
FIG. 2. *Changes in quality measurements of biscuits containing various amounts of leavening agent when stored at 43.3° C. (110° F.).* X = *4 oz. sodium bicarbonate;* O = *10 oz. sodium bicarbonate;* ⊗ = *16 oz. sodium bicarbonate;* ⊠ = *22 oz. sodium bicarbonate;* ● = *4 oz. ammonium bicarbonate;* ■ = *4 oz. of each of sodium bicarbonate and ammonium bicarbonate.*

No conclusions could be drawn from pH measurements on the alcohol extracts and on extracts of the defatted biscuits in 10% sodium chloride solution. The trends here were quite different from those observed in the experiment with different packages. An initial rise in pH occurred that is at present unexplainable.

The pH measurements on 10% potassium chloride extracts of the whole biscuits followed trends similar to those previously observed. The rate of decrease in pH was greater for biscuits containing 16 oz. and 22 oz. sodium bicarbonate. Biscuits containing 10 oz. sodium bicarbonate and both sodium and ammonium bicarbonate behaved similarly and changed more slowly than the biscuits containing the higher concentrations of leavening agent. The biscuits containing 4 oz. of leavening agent changed slowly; those with 4 oz. sodium bicarbonate changing least of all.

Fluorescence measurements indicated greater changes at the higher soda levels, with biscuits containing ammonium bicarbonate behaving similarly to biscuits containing 10 oz. sodium bicarbonate. There was a more rapid increase in fluorescing substances in biscuits containing both leavening agents than in biscuits containing the smallest quantity of sodium bicarbonate. Both of these quality tests indicated that the lower the soda level the more desirable was the biscuit, both in initial quality and in maintenance of quality during storage.

The mean palatability scores obtained on the biscuits initially and after seven weeks' storage are given in Table V. Increasing soda level evidently resulted in a definite decrease in initial flavour score. Seven weeks' storage lowered the flavour ratings, with the exception of the biscuit containing ammonium carbonate, which did not change in score during this storage period. However, the use of ammonium carbonate either alone or in combination with sodium carbonate resulted in reduced initial palatability scores. The biscuit containing 22 oz. of sodium bicarbonate deteriorated significantly more than the other types of biscuit. These features were assessed statistically on duplicate trials by the taste panel.

TABLE V

MEAN PALATABILITY SCORES OF BISCUITS CONTAINING VARIOUS AMOUNTS OF LEAVENING AGENT, STORAGE AT 43.3° C. (110° F.)

Leavening agent		Palatability scores	
		Initial	At seven weeks
Sodium bicarbonate,	4 oz.	8.1	7.5
	10 oz.	7.7	7.1
	16 oz.	7.2	6.4
	22 oz.	6.8	5.2
Ammonium bicarbonate	4 oz.	6.3	6.4
Sodium bicarbonate	4 oz. }	7.3	6.8
Ammonium bicarbonate	4 oz. }		

From the above results, it is evident that sodium bicarbonate was a more desirable leavening agent, and that smaller quantities were most desirable.

Effect of Protein Components

The mean values and an analysis of variance of fluorescence measurements made on biscuits with different protein components are shown in Table VI. Measurements of pH were omitted because they were of little value in interpreting quality differences. The behaviour of the biscuits at 60.0° C. (140° F.) was somewhat different from that at the lower temperatures. The results at this high temperature show the wheat germ and soya flour biscuits to have generally lower fluorescence value, while at the lower temperatures only the

TABLE VI

FLUORESCENCE CHANGES IN BISCUITS WITH DIFFERENT PROTEIN COMPONENTS

A. Table of means

Variable under study	Fluorescence (photofluorometer units)				
	60.0° C. (140° F.)	43.3° C. (110° F.)	26.7° C. (80° F.) (16% R.H.)[3]	26.7° C. (80° F.) (85% R.H.)[3]	
Type of biscuit					
Skim milk powder	37.29	29.92	24.96	24.10[1]	29.03[2]
Wheat germ	32.35	27.90	27.13	26.30	26.94
Powdered egg yolk	43.05	40.45	40.31	36.97	38.40
Soya flour	31.16	29.60	28.53	31.03	33.03
Necessary difference	3.46	3.13	2.10		
Type of package					
300 *MST* cellophane	32.74	31.07	30.90	29.53	
Reynolds' metal *A*-10	36.77	32.85	30.56		31.65
Reynolds' metal and cellophane	38.38	31.98	29.23		27.64
Necessary difference	3.00	—	—		
Storage time					
Initial	26.28	26.28	26.28	26.28	26.28
1 day	30.26	30.89	—	—	—
3 days	31.65	30.60	—	—	—
7 days	35.17	28.14	29.24	27.44	28.50
15 days	40.72	—	—	—	—
21 days	—	30.70	28.25	29.29	31.59
31 days	42.02	—	—	—	—
49 days	—	30.22	29.18	32.08	30.69
105 days	—	34.55	30.51	—	29.15
217 days	—	38.66	33.98	—	36.42
Necessary difference	3.87	4.14	2.35		

[1] *Values in this column for biscuits packaged in 300 MST cellophane.*

[2] *Values in this column combined results for biscuits packaged in Reynolds' metal A-10 and in Reynolds' metal and cellophane.*

[3] *R.H. = relative humidity.*

TABLE VI—*Concluded*

FLUORESCENCE CHANGES IN BISCUITS WITH DIFFERENT PROTEIN COMPONENTS—*Concluded*

B. Analysis of variance

Variance attributable to:	60.0° C. (140° F.)		43.3° C. (110° F.)		26.7° C. (80° F.) (16% R.H)[3]	
	D.f.	Mean square	D.f.	Mean square	D.f.	Mean square
Package	2	336.81**	2	44.20	2	31.12
Biscuits	3	880.71**	3	1375.88**	3	1419.91**
Time	4	666.90**	6	295.33**	4	120.99**
Biscuits × package wrappers	6	84.14	6	183.92**	6	16.88
Package × times	8	65.73	12	22.32	8	25.02
Biscuits × times	12	121.29*	18	48.17	12	9.46
Biscuits × package × times	24	42.14	36	50.10	24	15.50
Between biscuits	60	23.81	84	26.25	60	17.36

* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% level of statistical significance.*

egg powder biscuit had high values. These measurements show cellophane, when used alone, to be a preferable wrap at the highest temperature, although it was definitely undesirable at the lowest temperature if the humidity was high. This undesirability was due to mould growth resulting from increased moisture contents of the biscuits rather than to changes detected by fluorescence measurements. No difference in packages was observed at 43.3° C. and 26.7° C. (110° and 80° F.) at low humidity. Fluorescing substances generally increased with time for all types of biscuits; although wheat germ biscuits reached a maximum value at 15 days' storage.

Initial palatability scores of biscuits with various protein components were: skim milk powder, 5.2; soy flour, 5.2; dried egg yolk, 5.5; and wheat germ, 6.0. Statistical analysis of the data indicated that the wheat germ biscuit was significantly better than the others.

Statistical analysis of the palatability scores on stored biscuits with various protein components (Table VII) lead to conclusions slightly different from those cited above. Wheat germ and soya biscuits were again most stable at 60.0° C. (140° F.) while the wheat germ biscuit was the most stable at 43.3° C. (110° F.) and 26.7° C. (80° F.) (16% relative humidity). Egg yolk biscuits and milk biscuits deteriorated most rapidly at the two higher temperatures. Cellophane was the most satisfactory packaging material at 60° C. (140° F.), no difference occurring at the lower temperatures unless the humidity was high. At 60.0° C. (140° F.) decrease in palatability of biscuits that were double wrapped was more rapid than for biscuits in either of the single wrapped packages. Highly moisture-vapour resistant wrappings caused more drastic reduction in palatability in biscuits containing milk powder when stored at the high temperature.

TABLE VII

PALATABILITY CHANGES IN BISCUITS WITH DIFFERENT PROTEIN COMPONENTS

A. Table of means

Variable under study	Palatability scores				
	60.0° C. (140° F.)	43.3° C. (110° F.)	26.7° C. (80° F.) (16% R.H.)[2]	26.7° C. (80° F.) (85% R.H.)[2]	
Type of biscuit					
Skim milk powder	3.4	4.6	5.5	4.1[1]	5.2[2]
Wheat germ	4.9	5.3	5.9	4.1	5.8
Powdered egg yolk	3.5	3.8	4.3	3.3	4.5
Soya flour	4.6	4.9	5.4	4.3	5.3
Necessary difference	0.26	0.21	0.17		
Type of package					
300 *MST* cellophane	4.4	4.7	5.2	3.9	
Reynolds' metal *A*-10	4.1	4.6	5.3		5.3
Reynolds' metal and cellophane	3.8	4.6	5.3		5.2
Necessary difference	0.23	—	—		
Storage time					
Initial	5.5	5.5	5.5	5.5	5.5
1 day	5.4	5.2	—	—	—
3 days	4.6	5.2	—	—	—
7 days	4.0	4.8	5.2	5.2	5.6
15 days	3.9	—	—	—	—
21 days	—	4.7	5.1	3.9	5.0
31 days	2.4	—	—	—	—
49 days	—	4.4	5.0	2.8	5.5
105 days	—	4.1	5.2	—	5.0
217 days	—	4.0	5.9	—	5.0
Necessary difference	0.29	0.27	0.19		

B. Analysis of variance

Variance attributable to:	60.0° C. (140° F.)		43.3° C. (110° F.)		26.7° C. (80° F.) (16% R.H.)[2]	
	D.f.	Mean square	D.f.	Mean square	D.f.	Mean square
Package	2	2.08**	2	0.10	2	0.04
Biscuits	3	8.35**	3	8.25**	3	7.49**
Time	4	14.61**	6	2.69**	4	1.48**
Biscuits × package	6	0.53**	6	0.22	6	0.16*
Package × times	8	0.38**	12	0.08	8	0.08
Biscuits × times	12	0.44**	18	0.35**	12	0.06
Biscuits × package × times	24	0.12	36	0.11	24	0.05

[1] *Values in this column for biscuits packaged in 300 MST cellophane.*

[2] *Values in this column combined results for biscuits packaged in Reynolds' metal A-10, and in Reynolds' metal and cellophane.*

R.H. = *relative humidity.*

* *Exceeds 5% level of statistical significance.*

** *Exceeds 1% level of statistical significance.*

Unfortunately the skim milk powder biscuit had a moisture content of 7.6% instead of the 6% or less required. The foregoing data on moisture levels (Tables II, III, and IV) permitted some estimation of the error introduced by this difference. Calculations indicated that the difference in moisture contents of the wheat germ and skim milk powder biscuits was not great enough to account for the poorer storage life of the latter material. Therefore, it is believed that the validity of the conclusions from this portion of the investigation will not be vitiated by the higher moisture content of the skim milk powder biscuit.

From considerations of both initial palatability and keeping quality, wheat germ biscuit was the most desirable source of protein. Storage at 26.7° C. (80° F.) in a dry atmosphere induced little change and single wrappings of moisture-resistant packaging materials appeared to be the most desirable for general use.

Relation between Palatability and Objective Measures of Quality

The palatability test, on the whole, was the most satisfactory measure of quality of the biscuits, but like most organoleptic tests the error of replication was high. There was, furthermore, a noticeable tendency for the scores to increase toward the end of the experiment, as can be seen in Table VII.

To assess the merits of the various objective tests correlation coefficients were calculated. These were limited to calculations on palatability–fluorescence, and flourescence–pH relations. As mentioned previously, calculations on peroxide oxygen measurements would have contributed nothing. Correlations between the various pH measurements were so small that calculations were useless. In no instance were related data on pH measurements and palatability available.

For each type of biscuit correlations between fluorescence values and palatability scores were found to be negative, highly significant, but generally low in magnitude, e.g. skim milk powder biscuit, − .687**; wheat germ biscuit, − .315**; dried egg yolk biscuit, − .386**; and soy flour biscuit, − .528**. If the data for any one variable were averaged over all others high correlations between fluorescence and palatability were obtained, e.g. for time periods, − .897; wraps, − .956; biscuit types, − .828; and for storage conditions, − .854.

Correlations between fluorescence values and pH measurements were calculated for the skim milk powder biscuit. Again, the correlations were low; − .245*, − .082, and − .088 for fluorescence with pH's of alcohol extracts, sodium chloride extracts, and potassium chloride extracts respectively. Averaging the data for all variables at each time period showed higher correlations, − .675, − .627, and − .614 between fluorescence and the respective extracts.

* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% level of statistical significance.*

While none of the quality measurements correlated closely with palatability scores, the fluorescence measurement reflected storage treatment with a fairly high degree of accuracy.   On the basis of the present experiment fluorescence measurements supported by palatability evaluation of initial and final samples would probably give a satisfactory picture of the stored biscuits.

Moisture Changes During Storage in Substitute Containers

The average moisture gain in grams is given in Table VIII for biscuits packaged as described for the preliminary experiment, where an average weight of 74 gm. per package of skim milk powder biscuits was stored at 26.7° C. (80° F.) and at 85% relative humidity.   The dimensions of the finished package were 2 in. × 2 in. × 2 in.   It is evident that 300 *M.S.T.* cellophane is unsatisfactory under these conditions and Reynolds' metal *A*-10 permits a small moisture increase.   This small moisture increase did not appear to affect the quality of the biscuits in the package (Fig. 1).

TABLE VIII

MOISTURE GAIN OF SKIM MILK POWDER BISCUITS PACKAGED IN VARIOUS MATERIALS AND STORED AT 26.7° C. (80° F.) AND 85% RELATIVE HUMIDITY

Type of package	Gain per package (gm.) after storage for:				
	2 wk.	4 wk.	6 wk.	8 wk.	16 wk.
300 *MST* cellophane	2.93	4.97	7.39[1]	—[2]	—[3]
Reynolds' metal *A*-10, with and without 300 *MST* cellophane liner	0.14	0.28	0.32	0.44	0.57
Tin-plate, with and without 300 *MST* cellophane liners	0.02	0.01	0.02	0.03	0

[1] *Some mould growth on biscuits.*
[2] *Mould growth too heavy to permit measurement.*

The second experiment included only 300 *M.S.T.* cellophane and Reynolds' metal *A*-10 packages, the latter with and without cellophane inner liners. The moisture gain for an average weight of 132 gm. of biscuit per package of dimension 2 in. × 3 in. × 3 in. is given in Table IX.   One outstanding feature of these results is that the inner cellophane liner contributed additional moisture protection; this was not evident from the earlier experiment. Little information can be gathered from the breakdown of the data into biscuit types, although there is some evidence that the package containing biscuits made with powdered egg yolk picked up moisture at a slightly faster rate than the others.

It was observed during these storage trials that the random sampling technique used in selecting these packages could still be applied at the 15-wk. samplings.   However, at the 31 wk. period it was no longer applicable to the packages stored at 26.7° C. (80° F.) and 85% relative humidity as a result of failure of the packaging material.   Under this condition of high humidity

TABLE IX

MOISTURE GAIN OF ALL TYPES OF BISCUITS PACKAGED IN VARIOUS MATERIALS AND STORED AT
26.7° C. (80° F.) AND 85% RELATIVE HUMIDITY

Variable under investigation	Gain per package (gm.) after storage for:				
	1 wk.	3 wk.	7 wk.	15 wk.	31 wk.
By package types					
300 *MST* cellophane	4.81	8.84	14.72	—[1]	—[1]
Reynolds' metal *A*-10	0.46	0.50	0.75	1.21	2.34
Reynolds' metal with cello-					
phane liner	0.40	0.43	0.64	1.07	2.01
By biscuit types[2]					
Skim milk powder	0.38	0.39	0.58	0.98	1.77
Wheat germ	0.41	0.46	0.66	0.83	2.56
Powdered egg yolk	0.50	0.52	0.98	1.89	2.34
Soy flour	0.42	0.48	0.54	0.87	1.85

[1] *Mould growth too heavy to permit measurement.*
[2] *Mean values for samples in Reynolds' metal A-10 with and without cellophane liner.*

some separation of layers of the Reynolds' metal wrapper had occurred with resulting loss in protection. This is supported by the data for packages containing wheat germ and soy flour biscuits: even though only apparently undamaged packages were chosen for measurements at this last sampling, the moisture gain during the last 16 wk. was higher than would have been expected from the data for the first 15 wk. At the same temperature, and 16% relative humidity many packages made from these substitute materials were destroyed by crickets, which infested the storage room during the 15 to 31 wk. interval. Again, the final measurements were made only on undamaged packages.

Changes in moisture content of the biscuits were measured during the course of the experiment on protein constituents. The only significant changes in moisture content occurred in cellophane wrapped biscuits stored at 60.0° C. (140° F.), and at 26.7° C. (80° F.) and 85% relative humidity. At the higher temperature the average moisture content for all types of biscuits was reduced from 5.8% to 2.7% in 15 days; while at 26.7° C. (80° F.) and 85% relative humidity the average moisture content for all types of biscuits increased to 12.8%.

Acknowledgments

References

1. MARKLEY, M. C. and BAILEY, C. H.   Cereal Chem. 8 : 29-38.   1931.
2. McKIM, E. and Moss, H. V.   Cereal Chem. 20 : 250-259.   1943.
3. PEARCE, J. A.   Can. J. Research, C, 21 : 57-65.   1943.
4. PEARCE, J. A.   Can. J. Research, D, 21 : 98-107.   1943.
5. PEARCE, J. A.   Can. J. Research, F, 22 : 87-95.   1944.
6. PEARCE, J. A., THISTLE, M. W., and REID, M.   Can. J. Research, D, 21 : 341-347.   1943.
7. PEARCE, J. A., WOODCOCK, A. H., and GIBBONS, N. E.   Can. J. Research, F, 22 : 34-38.   1942.
8. SCHULERUD, A.   Cereal Chem. 9 : 128-136.   1932.
9. SHERWOOD, R. C., ANDREWS, J. S., WADE, W. B., and BAILEY, C. H.   Ind. Eng. Chem. 25 : 437-440.   1933.
10. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E.   Can. J. Research, D, 21 : 1-7.   1943.
11. THISTLE, M. W., REID, M., and GIBBONS, N. E.   Can. J. Research, D, 21 : 267-269.   1943.
12. WHITE, W. H.   Can. J. Research, D, 19 : 278-293.   1941.
13. WHITE, W. H. *et al.*   (In preparation.)
14. WHITE, W. H. and THISTLE, M. W.   Can. J. Research, D, 21 : 211-222.   1943.

DRIED WHOLE EGG POWDER

XVI. RELATIVE STABILITY OF POWDERS OF DIFFERENT QUALITY

BY M. W. THISTLE, W. HAROLD WHITE, D. A FLETCHER,
AND JESSE A. PEARCE

DRIED WHOLE EGG POWDER

XVI. RELATIVE STABILITY OF POWDERS OF DIFFERENT QUALITY[1]

By M. W. Thistle[2], W. Harold White[3], D. A. Fletcher[4],
and Jesse A. Pearce[2]

Abstract

When stored at 24° C. (75° F.) for one month, samples of dried whole egg powder of varying initial qualities, collected from 10 Canadian plants over a period of six months, developed fluorescent materials at much the same rate. Powders from two of the plants, heat-treated to provide good, questionable, and definitely poor samples, were stored at 24° C. for periods up to four months. Some of the observed differences in stability were attributable to different moisture contents; but within plants, good and poor quality powders deteriorated at the same rate. In a third experiment, mixtures of good and poor quality powders having a similar moisture content also deteriorated at the same rate. It is concluded that the rate of quality deterioration in egg powders, as determined by fluorescence changes, is independent of the initial quality.

Introduction

The question has sometimes been asked: do egg powders of varying initial quality deteriorate differentially? This has considerable practical significance in that if good quality powders deteriorate at a relatively rapid rate, some of the advantage of excellent initial quality might be lost during shipment or storage. Although information is available on the stability of egg powder (2, 3, 7, 8, 9), this particular point does not appear to have received detailed attention. The present study was designed to answer the question.

Materials and Methods

In these laboratories, during 1942, a survey was made of the quality of all the dried egg powder produced in Canada. At each egg-drying plant samples were taken from each carlot of powder intended for export to Britain, and their initial quality determined. Duplicates of certain samples were stored for a month at 24° C. (75° F.) in order to assess the relative stability of powder produced at different seasons and in different plants.

In studies on the effects of heat treatment it was found that dried egg was susceptible to damage by heat at even slightly elevated temperatures: samples from two Canadian plants were heat-treated for five days at 26.7°, 35.0°, and 43.4° C. (80°, 95°, and 110° F.), as described in a previous communication

(8). These heat-treated samples also were stored at 24° C. for periods up to four months in order to obtain information on their subsequent stability.

Further information was sought by measuring the relative stability of mixtures of poor and good quality material. Egg powder having a fluorescence value (4) of 60 was thoroughly mixed at 0, 1, 5, 15, and 30% levels with powder having a fluorescence value of 18. Uniform moisture contents (from 3.5 to 3.6%) were obtained in the mixed samples by permitting them to stand in a closed chamber at 4.4° C. (40° F.). Samples of the mixed powders were stored at 26.7° and 37.8° C. (80° and 100° F.) for periods of 3, 10, 17, 24, and 31 days. Initial fluorescence values of the treated powders ranged between 18 and 27, and were approximately proportional to the amount of poor powder added.

Quality was assessed by means of potassium chloride (6) and fluorescence values (4, 5, 6), chosen for present purposes as being most sensitive of the objective tests available. Indeed, the fluorescence test has been shown to be remarkably sensitive to heat damage in dried eggs (8) even at temperatures as low as −40° C. (7). Since moisture content has been shown to have important effects on the stability of dried egg (1, 2, 9) the total volatile content (6) was also noted.

Results

Survey Material

The results are presented in Table I. It is apparent that initial quality (as measured by fluorescence) was lower during July and August, the hottest months: the defect in commercial practice responsible for this lower quality was rectified in 1943 by the installation of devices for cooling the powder as it left the drier. Stability was poor in July (average of only five samples) but otherwise it appears that seasonal samples deteriorated at about the same rate under the storage conditions used. Also samples from different Canadian plants all deteriorated at about the same rate.

The influence of moisture content, under these conditions, was rather small.

The potassium chloride values, while more variable than the fluorescence measurements, present essentially the same information.

Heat-Treated Samples

The results are shown in Fig. 1.

The powder from Plant I deteriorated at a slower rate than that from Plant II: this difference is considered to be due primarily to the fact that the powder from Plant I contained 3.5% moisture while that of Plant II contained 5.6%. It has been shown that the stability of egg powder decreases with increase in the moisture content (9).

However, within plants the curves for good, intermediate, and poor powders possess approximately the same slope, indicating that if a uniform moisture content is attained, all powders in the edible range of quality deteriorate at about the same rate.

TABLE I

Mean values for quality measurements of dried egg samples from all Canadian
plants, collected from July to December, 1942, before
and after storage at 24° C. for one month

	No. of samples	Moisture content, %	Potassium chloride value, %		Fluorescence value, units	
			Initial	Final	Initial	Final
Means, averaged over all plants						
Month of collection						
July, 1942	5	4 6	66	50	30	46
August	29	4.3	65	53	29	35
September	30	4.6	67	54	23	35
October	30	4.0	74	62	22	30
November	26	4.0	70	62	22	32
December	30	3.8	69	62	23	29
Means, averaged over all sampling times						
Plant No.						
1	25	4.5	68	57	25	33
2	20	3.9	74	62	22	30
3	11	3.8	73	66	20	26
4	2	5.0	63	51	26	36
5	24	4.3	68	52	29	40
6	11	4.4	70	65	21	31
7	40	4.2	64	53	24	32
8	10	3.2	74	67	21	30
9	6	4.5	78	67	19	30
10	2	4.9	73	73	22	31
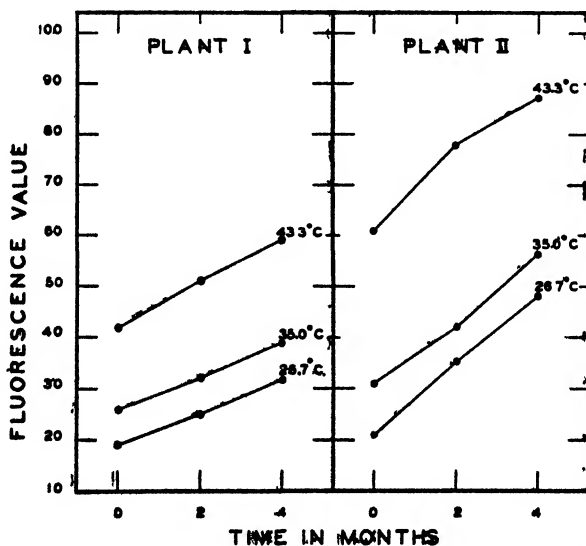
Fig. 1. *Relative stability of heat-treated egg powders stored at 24° C.*

Mixtures of Poor and Good Powders

The results are plotted in Fig. 2. The shapes of the curves are similar, indicating that samples with differing initial quality all exhibited similar behaviour under accelerated storage conditions. The small differences in slope were subjected to statistical analysis to ascertain their significance. Table II shows that the interactions between initial quality and storage time, and between initial quality and storage temperature, both failed to attain statistical significance. Hence the addition of low quality powders had no demonstrable effect on the rate of deterioration.
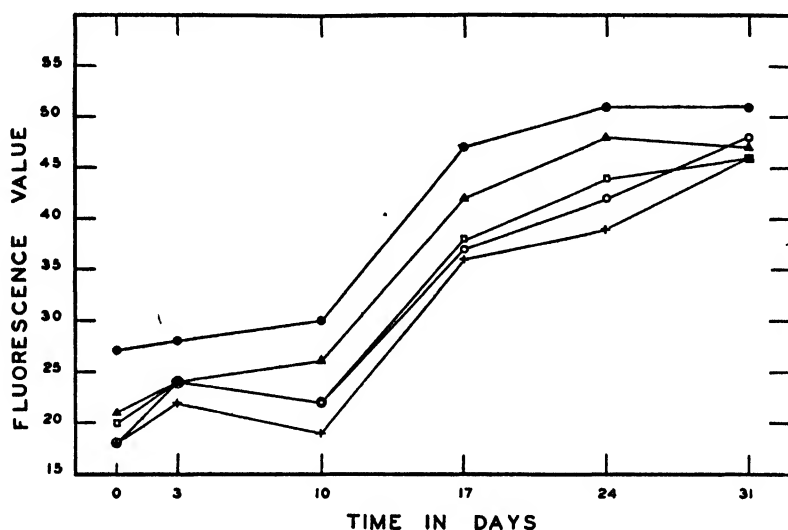
FIG. 2. *Relative stability of mixtures of poor and good quality egg powders, averaged over storage temperatures of 26.7° and 37.8° C. Percentages of poor powder added:*
$+ = 0$; $\bigcirc = 1$; $\square = 5$; $\blacktriangle = 15$; $\bullet = 30$.

TABLE II

ANALYSIS OF VARIANCE OF THE RELATIVE STABILITY OF MIXTURES OF
POOR AND GOOD QUALITY EGG POWDERS

Source of variance	Degrees of freedom	Mean square
Initial quality × time	16	11.4
Initial quality × temperature	4	9.4
Initial quality × time × temperature (error)	16	9.8

Conclusion

The evidence presented shows that when the moisture content was similar, good quality egg powders did not deteriorate at a faster rate than powders of lower quality in the edible range.

References

1. BROOKS, J. J. Soc. Chem. Ind. 62 : 137-139. 1943.
2. HAWTHORNE, J. R. J. Soc. Chem. Ind. 62 : 135-137. 1943.
3. KLOSE, A. A., JONES, G. I., and FEVOLD, H. L. Ind. Eng. Chem. 35 : 1203-1205. 1943.
4. PEARCE, J. A. and THISTLE, M. W. Can. J. Research, D, 20 : 276-282. 1942.
5. PEARCE, J. A., THISTLE, M. W., and REID, M. Can. J. Research, D, 21 : 341-347. 1943.
6. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E. Can. J. Research, D, 21 : 1-7. 1943.
7. THISTLE, M. W., WHITE, W. H., REID, M., and WOODCOCK, A. H. Can. J. Research, F, 22 : 80-86. 1944.
8. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 194-202. 1943.
9. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 211-222. 1943.

PACKAGING

I. WATER-VAPOUR RESISTANCE OF CELLULOSE-BASE CONTAINERS[1]

By A. H. Woodcock[2], Marion G. Chapman[3] and Jesse A. Pearce[4]

Abstract

Lacquers and resins coated on paper stocks were less effective water-vapour barriers than waxes.   Wax coatings became more effective as the density of the base stocks increased.   A flexible wax compound at 40 lb. per ream of 500 sheets, 24 by 36 in., was found most satisfactory for coating "Cellophane."

Rectangular containers were found more suitable than cylindrical containers. Wax dipping followed by an overwrap produced water-vapour–resistant packages, but the bag-in-box type, using wax-coated Cellophane as liner, was also effective. Pouch-type liners were favoured.

Bag-in-box type packages using wax-coated Cellophane as the liner bag were developed for use on dried egg powder, and a wax-impregnated, wax-coated outer container was devised for Army Mess Tin Ration Kits.

Mean moisture gain plus twice the standard deviation was used as a merit factor in distinguishing between packages.

Introduction

Shortages of tin plate, rubber, and shipping space have increased the importance of dehydrated foods.   Dried foods generally keep better if their moisture content is maintained at or near the original low level: this raised the problem of suitable containers for their protection, and reasonably water-vapour–resistant packages had to be provided from available packaging materials, such as paper products and waxes.   The urgency of war demanded an early solution of the problem.

Other factors were considered but the present study deals primarily with water-vapour permeability (W.V.P.).   Tests on a wide variety of commercially available materials, as received, after folding, and when made into packages, resulted in observations on materials and package design that are believed to be of some general value and are therefore reported here.   Since only comparative values of currently used stocks are of practical concern, no attempt has been made to convert results to unit thicknesses of base stock or coatings.

Methods

A vapometer method of measuring W.V.P., while none too reliable (1), provided simple and rapid comparative measurements on the various stocks,

both as received and after folding through 180° over the rounded edge of a metal plate 1 mm. thick. Two folds at right angles were made. Thus the better types of water-vapour barriers could be selected for fabrication into experimental packages.

Cylindrical and rectangular packages, each containing 60 gm. of calcium chloride, were placed in a test chamber operating at 80° F. and 85% relative humidity, resulting in a vapour pressure difference (V.P.D.) of 22.4 mm. of mercury. W.V.P. was measured by following the gain in weight. Gains are recorded as the averages of six packages of each type. Dummy packages were included in order to correct for sorption by the packaging materials.

Since one phase of the investigation was concerned with the development of a package suitable for dried egg, further tests were made on packages filled with dried egg powder and stored in the test chamber (V.P.D. approximately 14 mm. of mercury).

Another phase of the investigation dealt with a container for Army Mess Tin Ration Kits: a suitable moisture barrier had to be devised for the outside of the package, since some of the contents is packed in tin plate that might destroy an inner barrier.

Merit Factor

The use of mean moisture gain as a measure of package merit is not altogether satisfactory, since 50% of the packages can be expected to gain more moisture than represented by the mean value. The maximum moisture gain is also a poor criterion of package quality since it is usually dependent on a single package. A value descriptive of the great majority of the packages was felt to be desirable, i.e. the mean plus some measurement of variability. In consequence a numerical value, "merit factor," has been used in these laboratories as a criterion of package quality.

The merit factor is defined as the mean plus twice the standard deviation. Theoretically, for large normally distributed populations, 2.5% of the members should exceed this value. Since the number of packages studied is usually small, the merit factor would probably be less reliable than this. In 281 measurements, 11 packages (4%) exceeded the merit factor; in an additional 100 measurements, 5% exceeded the merit factor. Both curves indicated approximately normal distribution. Hence the merit factor might be used as an indication of the protection afforded by approximately 95% of the packages when small numbers are considered.

The merit factor might be used as a standard of reference for commercially available package types if standardized for temperature, water-vapour pressures, size of package, and quantity of hygroscopic material, e.g., calcium chloride. Certain limitations would have to be considered, such as the possibility of badly skewed curves.

Results

W.V.P. of Various Packaging Materials

The results are given in Table I. In general wax-impregnated krafts were less effective than wax-coated krafts; lacquers and resins provided about equal protection and were less effective than waxes; and wax coatings became more effective as the density of the base stocks increased. In the denser stocks, e.g., "Cellophane", wax coating on one side was approximately as effective as wax coatings on both sides and as effective as laminated Cellophane. A commercial firm offered the flexible wax compound found most satisfactory for coating Cellophane (40 lb. of wax per ream of 500 sheets , 24 by 36 in): the term "wax-coated Cellophane" will indicate use of this flexible preparation. W.V.P. measurements after folding showed increases ranging from 1/5 to 60 times depending on the type of material under study.

W.V.P. of Fabricated Packages

The results are shown in Table II, and in general support the conclusions drawn from Table I. However, it was found that even under the best conditions, the W.V.P. of completed packages was often 2 to 10 times as high as that of the same material in sheet form.

Of the wax-coated cylindrical containers, the fibre-bodied type was more effective than the all-fibre type. Fibre-bodied containers suffered from failure at the metal-to-fibre seam, and in the all-fibre type it was difficult to obtain a tight closure when inserting the friction-plug lid. Wax-dipping after fabrication improved the fibre-bodied type.

Although cylindrical containers are stronger than rectangular containers, they occupy about 20% more space; however, the relative weakness of rectangular packages can be compensated for by enclosure in a suitable master carton.

Considering rectangular containers, it is evident that an overwrap further reduced the W.V.P. of wax-dipped packages by approximately 50%. The bag-in-box type also produced effective packages. Of the more effective liners, wax-coated Cellophane was preferred since the cost of a second sheet was avoided. *

No great difference in effectiveness was evident between wedge and pouch type liners. However, it is considered that there is a danger of leaks developing at the seal around the gusset in the wedge type. Therefore it was believed desirable to use pouch type liners (3).

Packages for Dried Whole Egg Powder

A number of package types were tested, using dried egg powder as hygroscopic material (Table III). In addition, differences between and within firms preparing these packages are considered in Table IV, together with an estimate of resistance to handling, i.e., after dropping the packages six times from a height of 30 in. to a cement floor. From consideration of Tables III and IV, and for reasons described elsewhere (3), choice was made of the

TABLE I

WATER-VAPOUR PERMEABILITY OF FILMS OF VARIOUS PACKAGING MATERIALS AT ROOM TEMPERATURE AND A VAPOUR PRESSURE DIFFERENCE OF APPROXIMATELY 26.3 MM. MERCURY

Material		None	Impregnated Wax	Lacquer	Resin	Asphalt	Wax, one side	Wax, two sides
				Surface coated				
				Water-vapour permeability, gm./sq. m./day				

(a) Single Sheets

Material		None	Impregnated Wax	Lacquer	Resin	Asphalt	Wax, one side	Wax, two sides
Stock								
60 Lb. kraft	plain	—	742 to 48*	—	28–34	7.0	15– 15–17–19	0.52–0.54–2.2
	folded	—	—	—	54–57	11	130–200–43–	26 – 33 – 59
30 Lb. sulphite	plain	—	—	25–46–52	—	—	–26	—
	folded	—	—	—	—	—	14–57	—
25 Lb. glassine	plain	—	—	45–97–140	34	—	4.0–4.3– 7.6	4.0
	folded	—	—	—	—	—	–8.4–16	—
300 M.S.T. Cellophane	plain	14	—	—	—	—	1.2–2.3–4.2	2.9
	folded	—	—	—	—	—	–2.6–8.4	3.3
450 M.S.T. Cellophane	plain	—	—	—	—	—	0.74	—
Pliofilm	plain	1.8	—	—	—	—	—	—
	folded	1.9	—	—	—	—	—	—

(b) Laminations

Material		60 Lb. kraft	30 Lb. kraft	20 Lb. sulphite	"Grease-proof" paper	25 Lb. glassine	300 M.S.T. Cellophane	"Metal-coated" paper	Metal foil and M.S.T. Cellophane
					Combined with:				
					Water-vapour permeability, gm./sq. m./day				
Bleached Manila board	plain	7.4	—	—	32	8.3–11	—	—	—
	folded	—	—	—	49	—	—	—	—
60 Lb. kraft	plain	28–41–43–50†	—	—	—	20‡	—	—	—
	folded	48–	—	—	—	23	—	—	—
30 Lb. kraft	plain	—	3.2	1.5	—	—	—	—	—
	folded	—	11.0	4.0	—	—	—	—	1.4†
25 Lb. glassine	plain	—	—	—	13	1.6–3.1–3.3	—	—	—
	folded	—	—	—	18	5.5–3.7–	—	—	—
300 M.S.T. Cellophane	plain	—	—	—	—	14	1.9–2.9	2.0	—
	folded	—	3.3–3.3	4.3	—	23	—	6.4	—

* *Range found after examination of 19 types.*

† *Asphalt as laminating agent, and* ‡ *dextrin glue as laminating agent; all others, wax-base adhesives.*

. TABLE II

WATER-VAPOUR PERMEABILITY OF VARIOUS FINISHED PACKAGES SUBJECTED TO
80° F. AND A VAPOUR PRESSURE DIFFERENCE OF 22.4 MM. OF MERCURY

Description of package	Mean water-vapour permeability, gm./wk.
Cylindrical	
All-fibre, wax-coated inside and out; mean, two types (area of transmission, 38 sq. in.)	4.0
Fibre-bodied with metal ends, one end friction-plug type (area of transmission, 31.4 sq. in.)	
A. Untreated, 60 lb. kraft	9.5
B. 60 Lb. kraft and 25 lb. glassine	2.9
C. Asphalt-coated, 60 lb. kraft	1.2
D. Asphalt-laminated paper; mean, two types	1.2
E. Wax-coated, 60 lb. kraft; mean, three types	0.60
F. 60 Lb. kraft, wax-dipped after fabrication	0.25
Rectangular (area of transmission, 50.7 sq. in.)	
Wax-dipped	
A. Dipped once; mean, two types	0.21
B. Dipped twice; mean, two types	0.16
C. Dipped once, then wrapped in 60 lb. kraft; mean, two types	0.12
Wrapped	
A. 300 M.S.T. Cellophane	2.3
B. 300 M.S.T. Cellophane over bag-in-box with 300 M.S.T. Cellophane pouch type liner	0.90
C. 60 Lb. kraft, wax-coated both sides, ends redipped after fabrication	0.13
Bag-in-box (comparison of pouch and satchel liners)*	
A. Wedge (side seam, wax to Cellophane)	0.93
B. Wedge (side seam, wax to wax)	0.78
C. Pouch	1.00
Bag-in-box (liner, pouch-type)	
A. 30 Lb. vegetable parchment, wax coated	8.1
B. 40 Lb. bloodstock, wax coated	4.8
C. 300 M.S.T. Cellophane	2.3
D. Bleached, laminated, 45 lb. glassine, wax-coated	1.2
E. Laminated 300 M.S.T. Cellophane	0.41
F. 300 M.S.T. Cellophane laminated to 25 lb. glassine	0.16
G. 300 M.S.T. Cellophane wax-coated	0.091
H. 300 M.S.T. Cellophane laminated to "metal-coated" paper	0.070

* *Surface area of package approximately 140 sq. in.*

bag-in-box type utilizing wax-coated Cellophane as the liner bag. Two sizes of package were recommended for dried egg powder; a 14 lb. container for restaurant use and a 5 oz. household package. The W.V.P. of the 14 lb. carton when filled with egg powder has been measured as 1.68 gm. per wk. Performance of the 5 oz. carton can be seen in Tables III and IV.

Fourteen pound container. This had a Cellophane liner, pouch type, 23 in. deep by 18 in. wide (inside measurements) with a heat-seal on either side of from ¾ to 1 in. The bag was made of 450 M.S.Y.T. Cellophane, with a wax coat on the inside. This Cellophane bag was fitted inside a bag made of

TABLE III

WATER-VAPOUR PERMEABILITY THROUGH FINISHED PACKAGES CONTAINING 142 GM. (5 OZ.) OF DRIED EGG POWDER (MOISTURE CONTENT, 5%). SUBJECTED TO 80° F. AND 85% RELATIVE HUMIDITY (V.P.D., APPROXIMATELY 14 MM. OF MERCURY)

Description of package	Water-vapour permeability, gm./wk.
Single wax-dip	0.119
	0.077
Single wax-dip then wrapped in 60 lb. kraft	
Wrapped in 60 lb. kraft, wax-coated both sides; ends redipped and overwrapped in 60 lb. kraft	0.077
Single wax-dip then overwrapped with 60 lb. kraft, wax-coated both sides and ends redipped	0.077
Bag-in-box (pouch type liner) *A.* 300 M.S.T. Cellophane laminated to 25 lb. glassine	0.126
B. Duplex Pliofilm	0.119
C. Wax-coated M.S.T. Cellophane	0.080
D. 300 M.S.T. Cellophane, laminated to "metal-coated" paper	0.060

60 lb. M.F. kraft, satchel bottom style, to give a made-up bag 9 by 9 in. square and $14\frac{3}{4}$ in. high. These were then enclosed in a corrugated carton with inside dimensions 9 by 9 by 10 in. high made from 100% Fourdinier kraft, B-flute board, full meeting flaps, bursting strength 200 lb. Mullen test. The manufacturer's seal consisted of 2 in. Cambric type, since stitches might damage the liner.

Five ounce household package. This package was also of the bag-in-carton type: the liner was made of heat-sealed 300 M.S.Y.T. Cellophane with a wax coat on the inside. Outside dimensions were $5\frac{3}{8}$ in. wide by $6\frac{3}{4}$ in. high; inside dimensions were $4\frac{5}{8}$ in. wide by $6\frac{3}{4}$ in. high. This was enclosed in a carton of outside dimensions of 4 in. high by $2\frac{3}{4}$ in. wide by 1 15/16 in. deep. The carton was of flat folding type with overlapping long flaps, made from 0.020 in. board free from reclaim, good bender.

These two packages enclosed in suitable semi-master and master containers, have to date proved satisfactory for shipments of dried egg powder from Canada to Great Britain.

A Container for Army Mess Tin Ration Kits

Examination of the contents of the old-style kits after dropping tests showed that many of the packages contained therein had ruptured. From the data in Table II many of these inner packages were redesigned. Some of these have been described (2).

The ration kit contains tin plate packages, so that wax-coated Cellophane would not be satisfactory as a water-vapour barrier for the outer container.

· TABLE IV

WATER-VAPOUR PERMEABILITY OF FINISHED PACKAGES (FROM VARIOUS MANUFACTURERS)
CONTAINING 142 GM. (5 OZ.) OF DRIED EGG POWDER (MOISTURE CONTENT, 5%)
SUBJECTED TO 80° F. AND 85% RELATIVE HUMIDITY WITH AND WITHOUT
ROUGH HANDLING.   (V.P.D., APPROXIMATELY 14 MM. OF MERCURY)

| Description of package | Manu-facturer | Water-vapour permeability | | | |
| | | Without handling | | With handling | |
		Mean, gm./wk.	Merit factor	Mean, gm./wk.	Merit factor
Carton adhesively sealed, wax paper wrapped, heat-sealed, ends dipped in wax, and whole overwrapped with kraft	A	0.200	0.272	0.297	0.397
	B	0.259	0.407	0.354	0.544
	C	0.561	0.751		
	D	0.234	· 0.370		
	D	0.170	0.234		
Carton adhesively sealed, dipped in wax, and wrapped with kraft paper	A	0.085	0.141	0.161	0.233
	B	0.280	0.414	0.539	0.673
	C	0.168	0.350		
Carton adhesively sealed, dipped in wax, wax paper wrapped, heat-sealed, ends redipped, and the whole over-wrapped with wax paper	A	0.030	0.062	0.072	0.143
	B	0.095	0.215	0.234	0.352
	D	0.189	0.247		
Carton adhesively sealed, wrapped in heat-sealed wax-coated 300 M.S.T. Cellophane	A	0.055	0.089	0.088	0.156
	A	0.159	0.283		
Carton pouch liner, heat-sealed, of wax-coated 300 M.S.T. Cellophane	A	0.071	0.111	0.159	0.223
	A	0.185	0.241		
Rectangular fibre-coated composite can: metal ends, one with friction-plug lid; cardboard body; whole can dipped in wax after filling and closing	C	0.141	0.245		
	E	0.341	0.473		

Inspection of data in Table II indicated wax-coated kraft as a likely alternative. Several waxes and methods of application, and two types of base stock were studied: the results shown in Table V indicated original kraft stock to be satisfactory, and that the most suitable coating of those studied was wax B (50% paraffin, 50% microcrystalline or amorphous wax) applied by a special double dip, i.e., a dip at high temperature to impregnate the board, followed by a dip at lower temperature to apply a surface coat. Under the test conditions employed there was no difference between sprayed and dipped packages.

Exposure and dropping tests were made on the completed rations (Table VI). The results verified the choice made from Table V, and further indicated that the spray treatment offered more protection to the contents when the packages were dropped at low temperatures. A kraft carton was designed on the basis of these experiments: this carton has been fully described elsewhere (2) and to date has been quite satisfactory.

TABLE V

WATER-VAPOUR PERMEABILITY OF WAXED KRAFT CARTONS 6¼ BY 4⅝ BY 3¾ IN. (139.4 SQ. IN.)
CONTAINING CALCIUM CHLORIDE AND SUBJECTED TO 80° F. AND 85% RELATIVE
HUMIDITY (VAPOUR PRESSURE DIFFERENCE, 22.4 MM. OF MERCURY)

Stock material	Wax	Procedure	Water-vapour permeability, gm./wk.
Specially treated for wax dipping	A	Double dip	0.186
Specially treated for wax dipping	B	Double dip	0.088
Ordinary	A	Double dip	0.179
Ordinary	B	Double dip	0.062** (0.018)*
Ordinary	B	Special double dip	0.028**
Ordinary	B	Single dip	1.288
Ordinary	B	Single spray	1.309
Ordinary plus kraft wrap	B	Single spray	0.635
Ordinary kraft	C	Double dip	0.070 (0.032)*
Ordinary kraft	D	Double dip	0.534
Ordinary kraft	E	Unknown	7.4

* *Values after further tests using the small package with 50.7 sq. in. of transmitting surface.*
** *Best appearance after exposure to a temperature of −40° C.*

TABLE VI

WATER-VAPOUR PERMEABILITY OF COMPLETED ARMY MESS TIN RATION KITS SUBJECTED TO
80° F. AND 85% RELATIVE HUMIDITY (VAPOUR PRESSURE DIFFERENCE UNKNOWN)

Type of container	Water-vapour permeability, gm./wk.		
	As received	After exposure to −40° C.	After dropping four times at −40° C.
Old kit*	0.381	0.459	1.904
New kit, kraft wrapped followed by double wax dip	0.431	—	2.331
New kit, special double wax dip	0.497	0.438	1.039
New kit, double wax spray corresponding to special double wax dip	0.435	0.560	0.560

* *Smaller than new kit.*

Acknowledgments

References

1. CHARCH, W.·H. and SCROGGIE, A. G.   Paper Trade J. 101 : 201-209. 1935.
2. KENNEDY, G. E.   Can. Chem. Proc. Inds. 28 : 504-518. 1944.
3. WOODCOCK, A. H. and BARRY, S. C.   Food in Canada, 3 : 19-21. 1943.

PACKAGING
II. A CELLULOSE-BASE CONTAINER FOR MODIFIED VACUUM PACKING[1]

By A. H. Woodcock[2]

Abstract

Films of wax-coated laminated "Cellophane" have been shown to transmit carbon dioxide 25 times as rapidly as they transmit oxygen. Packages made from this film and gas-packed with carbon dioxide produce a vacuum pack on standing. Factory trials indicated that this type of packaging is feasible commercially, and shipping trials have shown the package to be reasonably substantial. Storage trials at 26.7° and 37 8° C. showed the package to be effective for a period of six months.

Introduction

The need for non-metallic food containers having low water-vapour permeability has been discussed in an earlier publication (10). Valuable constituents of many foodstuffs, carotene (4), ascorbic acid (1), butter fat (5), and other fats, are believed to be susceptible to deterioration in the presence of oxygen and to require protection from it. Complete protection can be obtained only by the use of packages made of material impermeable to oxygen and hermetically sealed. For this purpose, tin plate is one of the most suitable materials. The present shortages of materials and cost considerations have focused attention on non-metallic films with low oxygen permeability.

While the previous paper in this series was confined to a study of the effectiveness of non-metallic containers as water-vapour barriers, it was possible to utilize some of this information in the development of a package having both low water-vapour permeability and low oxygen permeability.

The present paper describes a non-metallic food container with low oxygen permeability believed suitable for packaging dried whole milk powders and other powdered products subject to oxidative deterioration.

Experimental

The apparatus used to measure the oxygen permeability of these films was similar to one described elsewhere (6) and is shown in Fig. 1. The film was placed between two hemispherical cups A and B. Both sides of the film were evacuated and the cup B was filled with gas at a few centimetres' pressure as measured by the manometer C. The vacuum pump was shut off from A by raising the mercury level above the point D, and gas diffused into A from B through the film. At regular periods the amount of gas in A was determined by raising the mercury level and compressing it into the calibrated
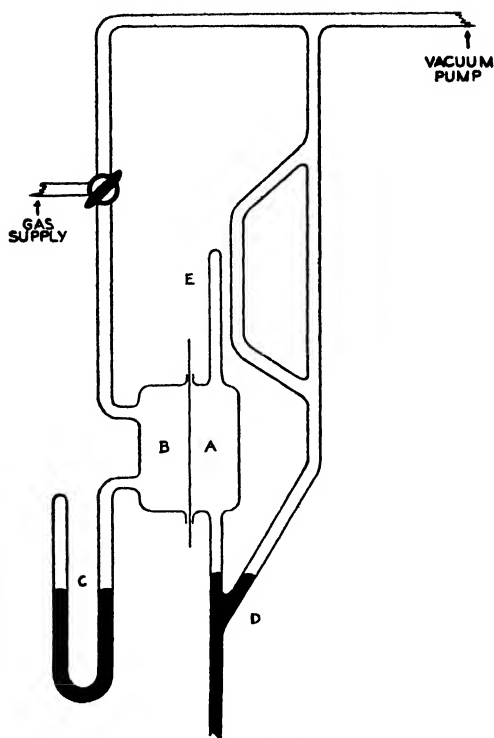
FIG. 1.   *Apparatus for gas permeability measurements*

tube E, where its volume and pressure were measured in a manner similar to that used with a McLeod gauge.   Compensation for pressure of volatile vapours, which reached saturation under compression, was made by taking a series of readings at different volumes.   Measurements at 24-hr. intervals permitted calculation of the permeability in absolute units.

Following development of a film with low oxygen permeability, packages were fabricated, filled with milk powder, sealed, and gas-packed.   Some of these were subjected to shipping trials, some to rough treatment at low temperatures, and others were put into storage at 26.7° and 37.8° C. (80° and 100° F.).

The storage experiment was designed to compare the quality of dried whole milk powder packed in air and packed in inert atmospheres using the current commercial method and these non-metallic containers.   One-pound tins were used for the commercial pack while 8 oz. and 20 lb. packages were used for the non-metallic gas-packs.   Milk powder quality was determined at three-month intervals by tasting tests (7).

Results

Description of Film Developed

After several unsuccessful trials a film with low oxygen permeability was designed.   This film consisted of two layers of 450 M.S.Y.T. "Cellophane"

laminated together with lacquer and coated on one side with a flexible wax compound (40 lb. per ream of 500 sheets 24 by 36 in.). The water-vapour permeability of this film when formed into a liner was found to be about one-eighth of that of the film used for the dried egg package (10).

Oxygen and Carbon Dioxide and Permeability of the Film

The oxygen and carbon dioxide permeability of this film as prepared is shown in Table I. After tests had been repeated several times on a sample of film, the permeability appeared to decrease; this change was believed to be due to the partial dehydration of the film when subjected to repeated evacuation. Drying a sample by subjecting it to vacuum at room temperature confirmed this (Table I). Furthermore, the ratio of permeability to oxygen

TABLE I

OXYGEN AND CARBON DIOXIDE PERMEABILITY OF LAMINATED 450 M.S.Y.T. CELLOPHANE, COATED ON ONE SIDE WITH A FLEXIBLE WAX COMPOUND

Condition of film	Penetration in ml. per sq. metre per 24 hr. per mm. pressure difference		
	Oxygen	Carbon dioxide	Ratio
As received	0.0075	0.186	24.8
Dried under vacuum	0.0036	0.091	25.2

and carbon dioxide was proportional, within the limits of experimental error, to the ratio of their solubilities in water at room temperature, i.e. 1 : 27, indicating that the Cellophane, which contains moisture, is the effective part of the film rather than the heat-sealing lacquer, laminating compound, or the wax coating. This had been observed previously for regenerated cellulose films (9); however, for films such as Pliofilm the ratio of permeabilities is believed to be about 1 : 4 (2).

Based on the values reported in Table I, a cubic package with 6 in. sides would transmit 60 ml. of oxygen per annum. Since this package should contain about 2500 gm. of milk powder, the amount of oxygen transmitted would be below the critical level associated with rapid fat deterioration in milk powders (5), if uniformly distributed through the contents. Should it react with the surface layer of milk, rancidity might occur, and the storage investigation was planned to compare the effectiveness of this method of gas-packing milk powders with the method currently in industrial use.

Packages of Low Oxygen Permeability

When a package prepared from this film is filled with carbon dioxide the partial pressure differential across the film is approximately 758 mm. of mercury since the partial pressure of carbon dioxide under normal atmospheric conditions is 2.28 mm. of mercury. It follows from Dalton's law that the

carbon dioxide will be lost from the package more rapidly than it can enter (3), and, since the diffusion rate of oxygen (Table I) in the reverse direction is much less, a partial vacuum is created. This was demonstrated by forming a pouch-type liner bag of this wax-coated laminated material over a paperboard frame and sealing. The packages were filled with carbon dioxide through a small hole (brogue hole), by evacuating in a chamber and replacing the air with carbon dioxide; the brogue hole was sealed with a drop of melted wax. On standing, carbon dioxide escaped and the bag collapsed as shown in Fig. 2. Calculations based on the results of Table I indicate that carbon dioxide would be lost at an initial rate of 14.1 cu. mm. per sq. cm. per day.
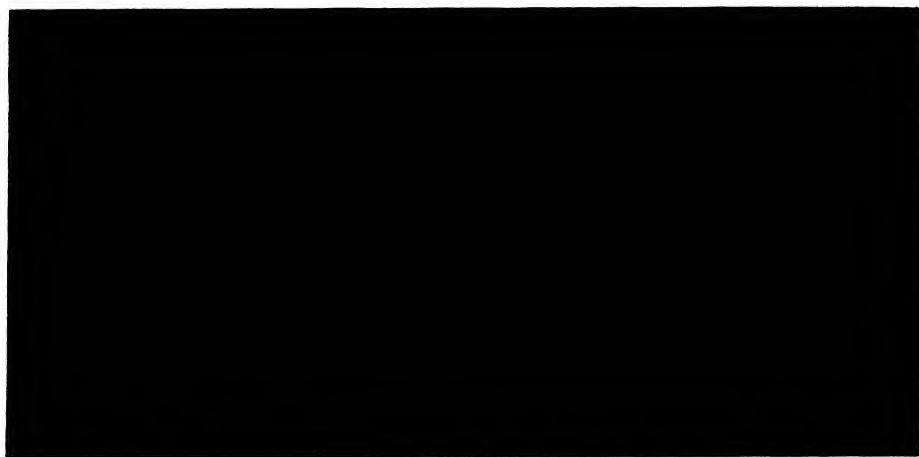
FIG. 2. *Comparison of package freshly filled with carbon dioxide and package after standing several days.*

A foodstuff packed in this manner was essentially vacuum-packed, since the carbon dioxide diffused in the atmosphere or was sorbed by the milk powder (8). Successful packs were therefore compressed into a firm block by atmospheric pressure and remained firm as long as the vacuum was maintained. A damaged package became soft again; this provided a simple test for faulty packs.

Tests

Dropping tests using packages containing 8 oz. of milk powder (30 in. on to a cement floor) were conducted at low temperatures. Two packages dropped at −40° C. (−40° F.) were fractured, one package out of two fractured at −15.6° C. (+ 4° F.), while both of two packages remained gas-tight at −22° C. (28° F.), indicating that at sharp freezing temperatures the package became too brittle to withstand rough handling.

A factory test was made with 50 packages of this type, each packed with 20 lb. of dried whole milk powder. The only extra equipment required was a mandrel and sealing irons. One package only was defective; this indicated reasonable commercial feasibility. Shipping tests were done with four of these

packages packed in a corrugated master carton. Packages were shipped by express, examined, and returned, then immediately reshipped by freight. On the return journey by freight one package broke down.

The results of the storage experiment are shown in Fig. 3; the experiment was concluded after nine months of storage since many of the flexible con-

EFFECT OF GAS-PACKING

EFFECT OF TEMPERATURE
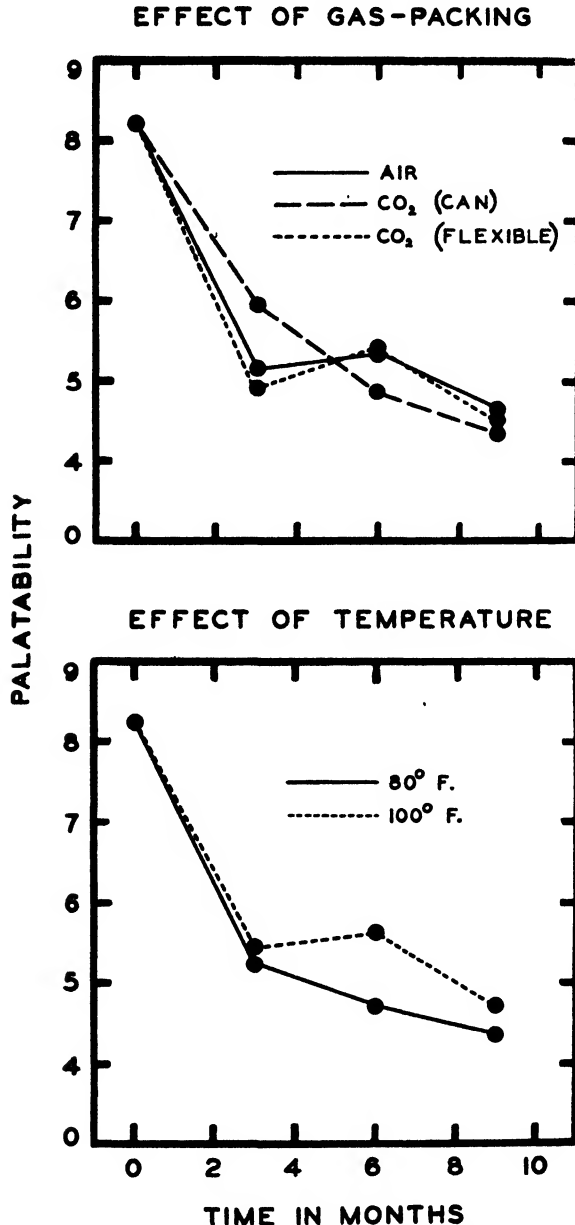
FIG. 3. *Effects of method of packing (average for both temperatures) and temperature (average for all methods of packaging) on the palatability of stored milk powder.*

tainers had lost their vacuum. Gas-packing by either method appeared to effect little improvement in the quality of stored milk powders. The better palatability in samples stored at temperatures of about 37.8° C. has been noted (7); both of these features are under further investigation (8).

Flexible packs were tested at each sampling time by their hardness. At six-month sampling all the non-metallic containers were still hard. At the nine-month sampling, all 8 oz. and 20 lb. containers at 37.8° C. were soft, while at 26.7° C. one of three 8 oz. and one of three 20 lb. containers were still hard. Alterations to extend the usefulness of this pack beyond this six-month limit are under investigation.

Acknowledgments

References

1. BORSOOK, H., DAVENPORT, H. W., JEFFREYS, C. E. P., and WARNER, R. C. J. Biol. Chem. 117 : 237-279. 1937.
2. ELDER, L. W. Modern Packaging, 16 : 69-72. 1943.
3. ELDER, L. W. Modern Packaging, 17 : 100-101. 1943.
4. KLOSE, A. A., JONES, G. I., and FEVOLD, H. L. Ind. Eng. Chem. 35 : 1203-1205. 1943.
5. LEA, C. H., MORAN, T., and SMITH, J. A. B. J. Dairy Research, 13 : 162-215. 1943.
6. MÜLLER, F. H. Physik. Z. 42 : 48-53. 1941.
7. PEARCE, J. A. Can. J. Research, F, 22 : 87-95. 1944.
8. PEARCE, J. A. Unpublished data.
9. SMITH, S. E. J. Elisha Mitchell Sci. Soc. 53 : 237. 1937.
10. WOODCOCK, A. H., CHAPMAN, M. G., and PEARCE, J. A. Can. J. Research, F, 23: 109-116. 1945.

RATION BISCUITS

II. EFFECT OF TYPE AND CONCENTRATION OF SHORTENING ON KEEPING QUALITY[1]

BY G. A. GRANT[2], J. B. MARSHALL[3], AND W. HAROLD WHITE[4]

Abstract

Ration biscuits prepared by two manufacturers and containing 8 to 23% of one compound animal–vegetable and three all vegetable shortenings were stored at 43.3° C. (110° F.) for 36 wk. Keeping quality was assessed by flavour, peroxide oxygen, and pH determinations.

The type of shortening was found to have a greater effect on keeping quality than the fat concentration or plant practice. Biscuits made with stabilized hydrogenated vegetable shortening were more stable than biscuits made with a compound animal–vegetable shortening. All biscuits became objectionable to the tasters after storage for 22 wk.

Introduction

Interest in the keeping quality and nutritional properties of ration biscuits has been stimulated by requirements of the armed services (6). The desirability of high fat biscuits as a means of increasing the caloric value of a ration must be considered in relation to the vulnerability of the shortening to oxidative decomposition when combined with other ingredients in a baked product (2). As far as can be ascertained there is little information available on the effect of concentration of shortening on the keeping quality of biscuits or crackers. This paper describes the results of an experiment in which the levels of four shortenings were varied in a simple formula, to determine the effect of the amount of each fat on its stability in a baked product.

Materials

The experimental material consisted of two lots of biscuits prepared independently by commercial manufacturers, using similar ingredients in a simple formula. Varying amounts of four shortenings drawn from stocks available in the biscuit plants were mixed with 50 lb. of soft wheat flour, from a common source, water added according to bakeshop practice, and soda used for leavening. Details of the proportions used are shown in Table I. The amounts of shortening used were calculated to give fat levels of 8, 13, 18, and 23% in the final product; reference to Table II shows that most of the fat was retained in the biscuits even at the highest levels.

TABLE I

BAKING FORMULAE OF BISCUITS MADE WITH FOUR SHORTENING LEVELS

Ingredients	Shortening levels			
	8%	13%	18%	23%
Shortening	4 lb. 6 oz.	7 lb. 8 oz.	11 lb.	14 lb. ½ oz.
Soft wheat flour	50 lb.	50 lb.	50 lb.	50 lb.
Water	17 lb.	15 lb.	13 lb. 8 oz.	11 lb. 8 oz.
Baking soda—				
Plant A	6 oz.	6 oz.	6 oz.	6 oz.
Plant B	8 oz.	8 oz.	8 oz.	8 oz.

TABLE II

MOISTURE AND FAT CONTENT OF BISCUITS MADE WITH FOUR TYPES AND LEVELS OF SHORTENING
IN TWO PLANTS

Shortening	Calculated shortening level, %	Fat and moisture content as determined experimentally			
		Plant A		Plant B	
		Fat, %	Moisture, %	Fat, %	Moisture, %
1	8	7.5	8.3	7.9	8.4
	13	13.3	7.5	13.0	7.2
	18	18.9	5.5	18.3	6.0
	23	23.3	5.1	21.0	5.3
2	8	8.4	8.0	8.1	8.8
	13	13.0	7.2	13.3	6.8
	18	19.6	5.7	17.7	5.9
	23	22.8	5.0	21.0	5.7
3	8	7.6	8.4	9.9	6.9
	13	12.8	7.2	15.6	6.1
	18	19.0	6.0	20.0	5.4
	23	21.7	5.3	22.9	5.8
4	8	7.5	8.2	7.6	7.7
	13	12.0	6.3	12.9	6.7
	18	18.2	5.4	18.3	5.6
	23	22.5	4.5	21.2	5.6

The shortenings were from commercial stocks available at the time the experiment was started, and included one compound animal–vegetable and three hydrogenated and stabilized vegetable products. Some analytical characteristics of samples drawn from the materials used are given in Table III.

Methods

The biscuits were stored at 43.3° C. (110° F.) in fibreboard containers lined with thin glazed paper, each box holding about 20 lb. The containers were overwrapped with waxed paper and finally with brown paper, these wrappings being carefully replaced after each sampling.

TABLE III

CHARACTERISTICS OF SHORTENINGS USED IN EXPERIMENTAL BISCUITS

Type of shortening	Swift stability time at 110° C. (hr.)	Characteristics				
		Saponi-fication No.	Iodine No.	Smoke point, °C.	Refractive index at 48° C.	Capillary melting point, °C.
1. Compound animal-vegetable	15	188.3	69.0	221	1.4580	49 0
2. Stabilized hydrogenated all-vegetable	20	188.6	67.3	213	1 4589	37 3
3. Stabilized hydrogenated all-vegetable	89	187.5	52.2	218	1.4565	40 3
4. Stabilized hydrogenated all-vegetable	43	191.3	62.9	215	1.4579	41.9

Quality was assessed on the whole biscuits by 16 tasters, who were required to score six samples in the morning and six in the afternoon on the following basis:— 10, excellent, fresh flavour and odour; 8, good, no off-flavour or odour; 6, fair, slight off-flavour or odour; 4, poor, marked off-flavour and odour; 2, very poor, offensive odour and flavour; and 0, inedible.

Peroxide values were determined by a method commonly used in these laboratories (7). The biscuits were ground with a rolling pin and fat extracted as follows:— Twenty grams of material was placed in a 100 ml. centrifuge tube, and 40 to 50 ml. of petrol ether (b.p. 30° to 50° C.) added; the mixture was thoroughly stirred with a power mixer and the extract decanted through a No. 4 Whatman filter paper into a 125 ml. Erlenmeyer flask. This procedure was carried out three times. Most of the petrol ether was then removed on a boiling water-bath and final traces by placing the flasks in a vacuum oven for about 30 min. at 40° C.

Preliminary experiments showed that sufficient fat for the analyses could be obtained by three extractions and that no appreciable difference in the peroxide oxygen values obtained resulted from increasing the number of extractions.

Measurements of pH were made on potassium chloride extracts of the ground biscuit material, using a 20 to 1 ratio of a 10% salt solution. The samples were weighed, salt solution added, and stirred four or five times before the supernatant solution was decanted for measurement of the hydrogen ion concentration. This procedure was rapid and facilitated handling large numbers of samples quickly and gave results that were comparable with those from more elaborate extraction methods.

The iodine numbers of the shortenings were determined by Kaufmann method (1, p. 432) and the saponification numbers by the official A.O.A.C. method (3).

Results

Flavour

Changes in mean flavour scores are shown in Fig. 1. While somewhat irregular, the general trends were similar throughout, and suggest that the highest concentrations of fat had a greater effect on the reaction of the tasters to flavour deterioration. There was also a difference between plants with respect to the flavour scores assigned to the biscuits of the same fat level. As was to be expected, the mixed animal–vegetable shortening showed the most rapid change.
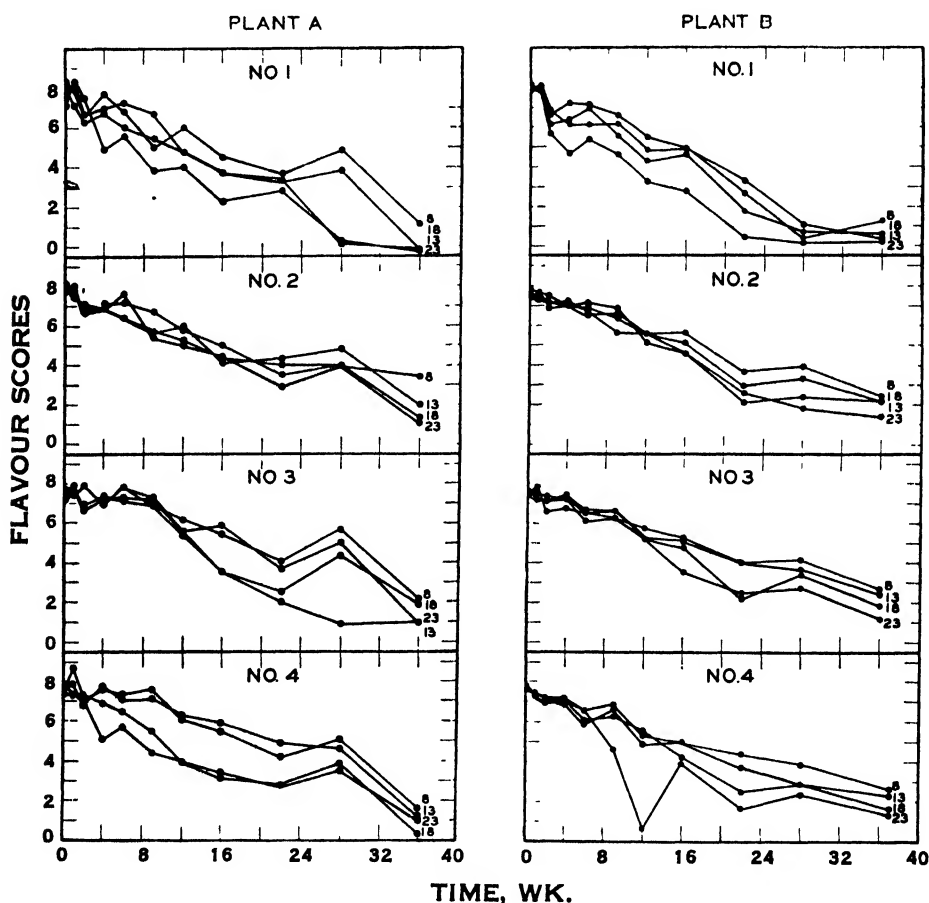
FIG. 1. *Effect of shortening concentration 8 to 23%, on flavour scores of ration biscuits stored at 43.3° C. (110° F.).*

Peroxide Oxygen

Increases in the peroxide oxygen values are shown in Fig. 2 for all of the variables tested. Differences between shortenings were more pronounced than the effects of amount of shortening or plant practice.

The shortest induction periods and greatest peroxide oxygen values were found in the material made from the compound animal–vegetable shortening, and the greatest stability with the hydrogenated and stabilized product, No. 3. Samples 2 and 4 gave indication of having reached the ends of their induction periods in the material from Plant A.
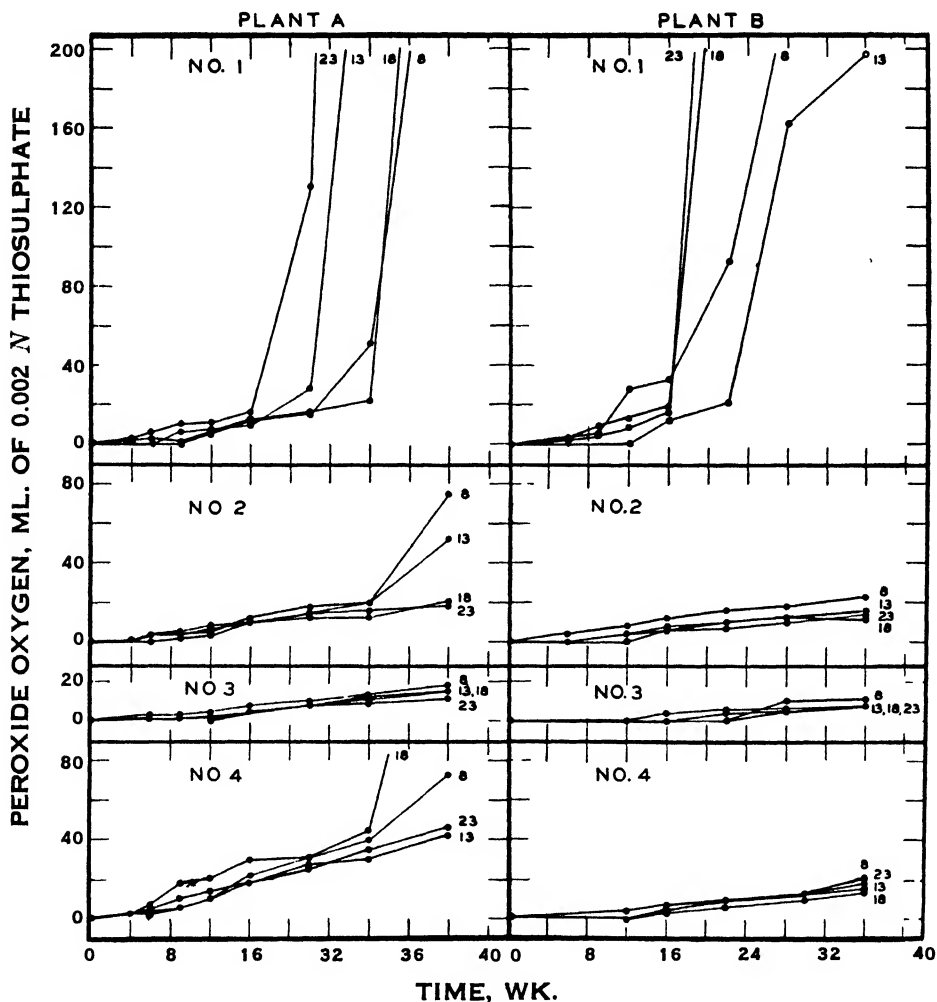
FIG. 2. *Effect of shortening concentration 8 to 23% on peroxide oxygen development in ration biscuits stored at 43.3° C. (110° F.)*

pH

The initial reactions of the test materials were quite alkaline and the changes shown in Fig. 3 gradual and somewhat erratic. No appreciable effect of shortening concentration was evident and only a slight indication of more rapid changes in the products from Plant A. The general trend was similar to that for flavour scores.
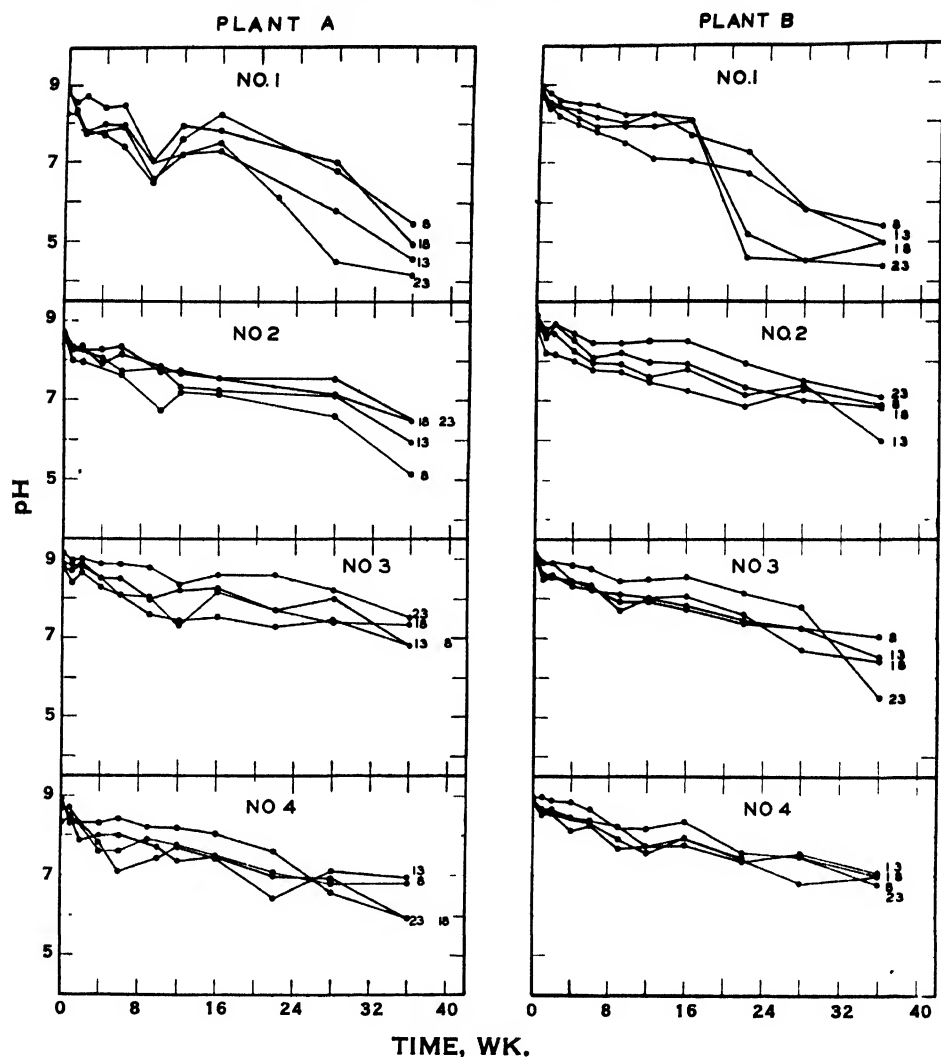
FIG. 3.   *Effect of shortening concentration 8 to 23% on pH values of ration biscuits stored at 43.3° C. (110° F.).*

Gradual increases in the acidity of stored flour and wheat germ have been observed (4, 5). Since the changes for material made with the mixed animal-vegetable shortening were greater and more rapid than those for the hydrogenated shortenings, the pH measurements appear to have reflected as well a change in the shortening that corresponds roughly to the end of the induction period.

Mean Values

Mean values for peroxide oxygen, flavour score, and pH are shown in Table IV, the data being averaged for the variables as indicated.

TABLE IV

THE EFFECT OF SHORTENING LEVEL, TYPE OF SHORTENING, PLANT PRACTICE, AND TIME OF
STORAGE ON THE DEVELOPMENT OF MEAN PEROXIDE OXYGEN, FLAVOUR, AND pH

Shortening level	8.0%	13.0%	18%	23.0%
Mean peroxide oxygen[1]	16.0	16.5	20.3	24.6
Mean flavour[1]	5.7	5.6	5.3	4.8
Mean pH[1]	7.3	7.5	7.7	7.8

Shortening	1	2	3	4
Mean peroxide oxygen[1]	56.0	11.6	3.1	6.7
Mean flavour[1]	4.8	5.4	5.6	5.6
Mean pH[1]	6.9	7.8	8.1	7.2

Plant	A		B	
Mean peroxide oxygen[1]	22.9		16.9	
Mean flavour[1]	5.5		5.2	
Mean pH[1]	7.3		7.8	

Time in weeks	0	1	2	4	6	9	12	16	22	28	36
Mean peroxide oxygen[1]	0	1	1	1	2	3	6	11	42	71	76
Mean flavour[1]	7.7	7.8	6.9	6.8	6.5	6.11	5.1	4.4	3.1	3.1	1.5
Mean pH[1]	8.9	8.5	8.2	8.0	8.1	7.8	7.8	7.8	7.2	6.9	6.1

[1] *Mean value over all other conditions studied for biscuit stored at 43.3° C. (110°F.)*

The differences resulting from the shortening levels were relatively small for all the measurements. The 23% level had the highest mean peroxide oxygen and lowest flavour scores. The apparently greater decrease in pH values at the lower shortening levels was due to the interaction of the levels with time and with shortenings.

There was little to choose among the shortenings except for shortening No. 1 (animal–vegetable), which yielded a biscuit with lower mean flavour and mean pH values and a much higher mean peroxide oxygen value than the other samples. The stability of the biscuits prepared with this shortening appeared to be markedly inferior to the others.

The magnitude of the differences occurring between plants was small. Plant B had lower mean peroxide oxygen and flavour and higher mean pH values.

An increase in mean peroxide oxygen content and a decrease in mean flavour and pH values occurred with time of storage. The change in mean peroxide oxygen value was greatest between storage for 22 and 36 wk., while the

largest change in mean flavour scores was evident between 9 and 36 wk. The changes in mean pH were gradual, with the greatest decrease resulting for shortening No. 1.

Analyses of Variance

The significance of the effects of the factors investigated was tested statistically by means of analyses of variance. Since the compound animal-vegetable shortening (No. 1) behaved quite differently from the others, the data pertaining to it were omitted from the computations with respect to peroxide oxygen. Moreover, as the changes in flavour score and peroxide oxygen values were small during the first stages of the experiment, only data for the period between 9 and 36 wk. were included in the statistical analyses.

Analyses of variance for flavour scores and peroxide oxygen values are given in Table V and for the pH data in Table VI. Differences among shortenings Nos. 2, 3, and 4 with respect to flavour were not significant and shortening level did not affect the peroxide oxygen values or the pH measurements significantly.

TABLE V

ANALYSES OF VARIANCE OF FLAVOUR AND PEROXIDE OXYGEN DATA FOR RATION BISCUITS STORED AT 43.3° C. (110° F.)

Source of variance	Degrees of freedom	Flavour value, mean square	Degrees of freedom	Peroxide oxygen value, mean square
Shortenings	3	12.58**	2	2726**
No. 1 vs. others	1	35.43**		
Others	2	0.57		
Shortening levels	3	19.26**	3	237
Linear regression on shortening levels	1	55.54**		
Time	5	88.68**	5	2537**
Plants	1	3.47**	1	5277**
Plants × time	5	3.34**	5	729**
Plants × shortening level	3	0.08	3	236
Plants × shortenings	3	0.15	2	1797**
Shortening level × shortenings	9	1.12*	6	272
Shortening level × time	15	0.54	15	122
Shortening × time	15	1.11**	10	373*
Time × No. 1 vs. others	5	2.09**		
Others	10	0.61		
Residual	129	0.48	91	177

* Indicates 5% level of statistical significance.
** Indicates 1% level of statistical significance.

Discussion

Increasing the fat concentration in ration biscuits did not significantly affect their stability as assessed by peroxide oxygen determinations and pH measurements, but as judged by flavour score the higher levels gave somewhat less acceptable products after storage at 43.3° C. (110° F.). Peroxides developed most rapidly and reached the highest concentration in the biscuits made with compound animal–vegetable shortening and this material also attained

TABLE VI

ANALYSIS OF VARIANCE OF pH DATA FOR RATION BISCUITS STORED AT 43.3° C. (110° F.)

Source of variance	Degrees of freedom	Mean square
Shortening levels	3	0.874
Shortenings	3	
No. 1 vs. others	1	17.590*
Others	2	1.654
Time	7	24.657**
Plants	1	2.655**
Plants × shortening levels	3	0.044
Plants × shortenings	3	0.890*
Plants × time	7	0.238
Time × shortenings	21	
No. 1 vs. others × time	7	3.024**
Others × time	14	0.051
Shortening levels × shortenings	9	
23% vs. others × shortenings	3	1.582**
Others × shortenings	6	0.2374*
Plants × time × shortenings	21	0.2351**
Residual	156	0.0954

** Indicates 1% level of statistical significance.
* Indicates 5% level of statistical significance.

the lowest rating in the organoleptic tests. All of the material had become objectionable after storage for 22 wk., off flavours and odours being detected before significant changes took place in some of the fats. Presumably this may have resulted from partial deterioration of the non-fat fraction. Variations in behaviour between plants may have been due in part to the difference in the amounts of baking soda used. This factor has been investigated in greater detail in these laboratories*.

On the basis of the present investigation, it is concluded that if a ration biscuit of high caloric value is required, fat levels could be safely increased by using stable shortenings without significantly altering the keeping quality of the biscuit.

Acknowledgments

References

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis. 5th ed. A.O.A.C., Washington, D.C. 1940.
2. BOHN, R. M. Oil and Soap, 13 : 302. 1936.
3. KAUFMANN, H. P. Studien auf dem Fettgebiet. Verlag Chemie, G. M. B. H., Berlin. 1935.
4. MARKLEY, M. C. and BAILEY, C. H. Cereal Chem. 8 : 29-38. 1931.
5. PEARCE, J. A. Can. J. Research, C, 21 : 57-65. 1943.
6. PEARCE, J. A. and MARSHALL, J. B. Can. J. Research, F, 23 : 22-38. 1945.
7. WHITE, W. H. Can. J. Research, D, 19 : 278-293. 1941.

* White, W. H. et al. Unpublished data.

DRIED MILK POWDER

I. METHODS OF ASSESSING QUALITY AND SOME EFFECTS OF HEAT TREATMENT[1]

By Jesse A. Pearce[2]

Abstract

The suitability of a number of objective tests of milk powder quality was assessed against subjective scores of palatability. The objective tests investigated were: oxygen and water sorption of the powders; chlorophyll and peroxide oxygen values of the fat; 'browning' of the powder; fluorescence values; changes in peroxidase, trimethylamine, volatile sulphur compounds, and diacetyl content; solubility by centrifuging and a potassium chloride solution method; titratable acidity; pH; congo rubin and iron numbers; foaming volume; coagulation by acid, alcohol, and rennet; dielectric constant; colour intensity and colour quality; refractive index; viscosity and surface tension. The subjective measurement of palatability was finally adopted as the most precise measure of milk powder quality.

While measurement of peroxidase activity was unsatisfactory in the determination of quality, the activity of this enzyme was observed to decrease with increase in time and temperature.

When palatability was used as a measure of quality, powders stored at 37.8° C. for seven days were preferred to powders stored at 26.7°, 48.9°, or 60.0° C. Interpretation of these results in terms of the temperature to which milk powder should be cooled indicated that 37.8° was the desirable temperature. Current commercial practice permits cooling to this temperature within a few minutes after the completion of drying.

Introduction

For a number of years it has been considered advisable to cool milk powders to room temperature immediately on completion of drying (13, p. 524). Drum-dried powders are believed to cool rapidly to this temperature, while spray-dried powders are often left in the drier for long periods, or packed without cooling. Spray-dried powders packed without cooling have been observed to have temperatures of 57° to 60° C. after 24 to 48 hr. (13, p. 521). However, no published information was found to indicate just how much deterioration may occur, or to what temperature it is desirable to cool the powder.

Data on dried whole egg powder show that the powder should be cooled to a temperature of 26.7° C. within three hours of its preparation if deterioration is to be prevented (21). The present paper is primarily concerned with the results of a similar experiment on spray-dried milk powder. However, before proceeding with the investigation some consideration was given to methods of measuring milk powder quality. Observations on these measurements are also recorded.

Tests of Milk Powder Quality

Subjective measurements of quality are generally affected by the interest and physical condition of the individual and even a large panel may vary in its average opinion from day to day. While individual tasters with great experience may apply reasonably uniform scores to food products that are tasted or smelled, their results may not conform closely to the opinion of the general public. Therefore, an objective measure of quality is to be preferred. To be satisfactory this objective measure must correlate closely with tasters' opinions. Since a great number of tests of milk powder quality are being used by various investigators, it was believed desirable to make a comprehensive survey of existing tests and to study other possible measures of quality. Existing tests have generally been used in studies where probably only one factor of milk powder deterioration was involved. Therefore these tests were evaluated on samples subjected to deterioration from several possible causes.

Material

Samples were obtained from six producers of spray-dried whole milk powder. From three of these, powder samples were supplied in August, September, October, 1943. The samples were divided into subsamples, some of which were tested without further treatment while others were assigned at random to a variety of storage conditions. These conditions were arranged to give different time of exposure to different temperatures at about 14° C. (25° F.) intervals from 0° to 60° C. (32° F. to 140°); to relative humidities of 16 and 85%; to atmospheres of air, nitrogen, and carbon dioxide; and to bright sunlight. Total number of samples tested was 38.

Methods Used for Testing Powder Quality

Two variations of the subjective method were used. One taste panel of 14 people scored the powders on the basis of 10 to 0, 10 being the equivalent of excellent fresh whole milk. The other panel, consisting of 10 people, scored on a basis of 3 to 0, 3 being the equivalent of excellent fresh whole milk.

The method of reconstituting milk powder was the same for both panels; 10 gm. of milk powder was mixed for 30 sec. in 80 ml. of distilled water at 38° C. (100° F.) in a Waring blendor and then chilled to 7° C. (45° F.) (11).

The objective tests included measurements of oxygen (10) and water sorption (15) of the powders. Changes in the fat were measured by determination of the chlorophyll value (4) and a colorimetric method for the determination of peroxide (3). The latter was chosen in preference to the iodometric method (16) commonly used since marked deteriorations occurred in milk fat before measurable values developed (18). Colorimetric measurements to detect 'browning' in the powder (7) and variations of the fluorescence measurement previously used (18) were also made. Changes in some components were measured by tests for peroxidase (6), trimethylamine (2), volatile sulphur-containing substances (19), and a test for diacetyl based on the oxidation of this component to acetic acid by hydrogen peroxide. Solubility was deter-

mined by a recommended method (1) and by the potassium chloride solution method used for dried whole egg powder (20). Titratable acidity (1) and pH were also determined. Colloidal characteristics, congo rubin numbers (12, pp. 58–63) and an iron number (9, p. 193), foaming volume (8), and coagulation by alcohol, by acid, and by rennet were also studied. Rennet coagulation was measured as the 'time of set' required for the sol–gel transformation of the reconstituted milk and rennet; time of set was determined by a 'tilted rod' method (14). Measurements were made of such physical characteristics as dielectric constant in a high viscosity, low dielectric oil (17, pp. 208-216), colour intensity and colour quality (22), refractive index, viscosity, and surface tension.

Results

Objective tests have been found by other investigators to be reasonably successful when the material was subjected to a single factor affecting quality. In the present work, it was believed desirable to test many factors simultaneously; under these conditions all the objective tests studied were unsuitable. Two of the tests, oxygen and water sorption, were found to be too cumbersome for routine control work and were omitted after a few trials. No method was found that would give an extract sufficiently clear for satisfactory refractive index measurements. With many of the tests, e.g. chlorophyll value, congo rubin, and iron numbers and coagulation by alcohol and acid, it was not possible to obtain a satisfactory end-point. Measurements of trimethylamine, of volatile sulphur-containing compounds, of coagulation by rennet, and of foaming volume were not easily duplicated. For many other tests, e.g. solubility by the potassium chloride solution method, pH, viscosity, surface tension, and dielectric constant, the error of duplication was equal to, or greater than, the difference due to quality.

Correlations of palatability with the remaining objective tests are shown in Table I; titratable acidity was significantly related to palatability although it was not a satisfactory method of predicting eating quality.

TABLE I

CORRELATIONS BETWEEN VARIOUS QUALITY TESTS ON SPRAY-
DRIED WHOLE MILK POWDER

Palatability, Panel I correlated with:	Correlation coefficient (36 D.f.)
Peroxidase value (5)	0
Colour intensity (22)	.06
Peroxide values (3)	−.11
Solubility index (1)	−.18
Colorimetric value (7)	−.20
Diacetyl value	−.23
Fluorescence (18)	−.26
Titratable acidity (1)	−.47**
Palatability, Panel II	.79**

' *Exceeds 1% level of statistical significance.*

It was possible that the palatability determinations lacked accuracy hence the testing by two different panels, scoring on two different scales. The correlation, shown graphically in Fig. 1, was reasonably close (Table I), although an error of about 35% was attributable to the tasters' judgments. Nevertheless, organoleptic determination of quality was a more precise measure than any objective test. Therefore, it was believed desirable to proceed with the investigation using the subjective test. However investigations of objective methods of measuring milk powder quality are continuing.
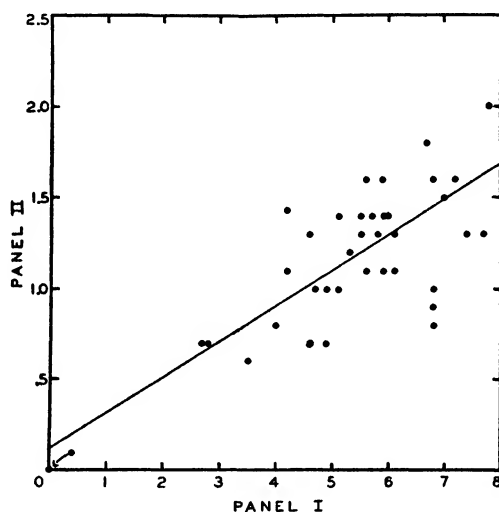
FIG. 1.   *Relation between the taste scores given by two panels to dried milk powders of varying quality.*

Effect of Heat-Treatment on Quality

While palatability scores by both panels (Table II) were considered the primary criteria of quality in this investigation, one of the other tests, the peroxidase measure, gave results believed worthy of some consideration. These results are also recorded (Fig. 2).

Materials and Methods

Spray-dried skim milk powder (1% fat), and whole milk powder (28% fat), prepared by a commercial processor, and an experimental lot of spray-dried whole milk powder (28% fat) were stored at 26.7°, 37.8°, 48.9°, and 60° C. (80°, 100°, 120°, and 140° F.) for seven days. The experimental powder was prepared using pasteurizing, condensing, and drying temperatures about 11° C. (20° F.) lower than those used by the commercial processor since it was felt that currently used temperatures may be too high to give an excellent quality of product.   Drying was done on a laboratory spray-drier (24).

Skim milk was reconstituted by mixing 10 gm. of powder with 100 ml. of distilled water and a palatability reference score of 10 indicated an equivalence to excellent fresh skim milk.

TABLE II

Table of mean values

Variable under study	Palatability score	
	Panel I	Panel II
Samples		
Skim milk powder (commercial)	4.0	0.6
Whole milk powder (commercial)	5.8	1.4
Whole milk powder (experimental)	6.0	1.5
Temperature (°C.)		
60.0	5.0	0.9
48.9	5.5	1.1
37.8	5.5	1.4
26.7	5.2	1.3
Time (days)		
Initial	5.3	0.7
1	5.0	1.2
2	5.6	1.0
3	5.1	1.0
4	5.2	1.3
5	5.4	1.2
6	5.2	1.2
7	5.0	1.3

Analysis of variance

Variance attributable to:	D.f.	Mean square	
		Panel I	Panel II
Samples	2	33**	3 7**
Temperature	3	1.3*	1.2**
Time	6	0.52	0.17
Samples × temperature	6	0.20	0.61**
Residual	66	0.44	0.078

* *Exceeds the 5% level of statistical significance.*
** *Exceeds the 1% level of statistical significance.*

The peroxidase value (6) was the time in seconds required for the fluorescence of the milk powder and test solutions to be blanked out by the development of the starch–iodine-blue colour. Milk powder (0.100 gm.) was thoroughly mixed in a fluorometer tube with 8 ml. of solution made from 20 ml. of 0.05 N sodium thiosulphate solution and 4.5 gm. of potassium iodide made up to 1 litre with acetate buffer (445 ml. of 0.1 N acetic acid and 545 ml. of 0.1 N sodium acetate solution). To this, 1 ml. of 2% starch solution was added, followed by mixing; the test-tube was then inserted into the photofluorometer and 1 ml. of 1.0% hydrogen peroxide added from a quick-flowing

blow-out type graduated pipette.   A stop watch was started immediately the
last drop of hydrogen peroxide was added and stopped when the photo-
fluorometer needle reached 10; the mixture was stirred throughout this period.
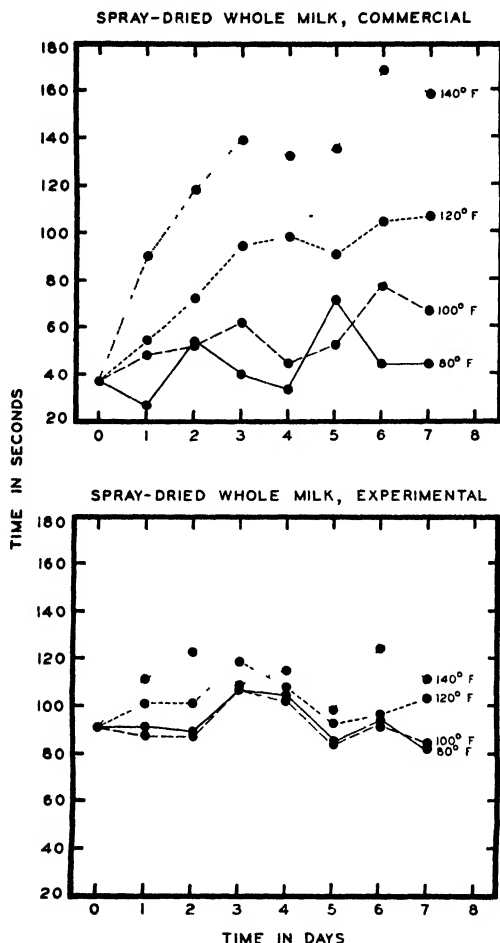
FIG. 2.   *Changes in peroxidase activity of commercial and experimental preparations of
dried whole milk powders stored for seven days at different temperatures from 26 7° to 60 0° C.
(80° to 140° F.).*

Results

· The results of the taste panel measurements are shown by a table of means
and an analysis of variance in Table II. A large difference was evident
between the quality of the whole and skim milk, the latter being almost
unacceptable to the taste panel.   This might be expected for two reasons;
the tasters were not accustomed to milk in this form and commercially dried
skim milk is usually prepared at temperatures still higher than those used
for whole milk.   The panel showed a slight preference for the experimental
material prepared at low temperatures, although this difference was not

statistically significant. Both panels preferred samples after storage at about 37.8° C. verifying previous similar observations (18, 23). The experimentally prepared powder stored at 60.0° C. and 48.9° C. was of lower quality than the commercially prepared sample and was of much higher quality at 37.8° and 26.7° C. This difference attained statistical significance as noted by Panel II. These results indicate that cooling the powder to 37.8° C. subsequent to drying is desirable. Since time effects were not significant (due probably to day-to-day variation in judgment of the tasters) it is difficult to specify any time within which cooling should be done. Work on dried egg powder indicated that cooling of that product should be done in less than three hours (21); current commercial practice has reduced this time to the order of minutes.

The low average score assigned initial powders by Panel II appeared to be significant. It would appear that even a day's storage at about room temperature improves the palatability of skim milk powders. This phenomenon is currently receiving attention in these laboratories.

Changes in the peroxidase value of dried whole milk powders are shown in Fig. 2. Although a constant peroxidase value had been observed in the initial experiments and a constant value was obtained for skim milk powders, the values for these whole milk powders showed a decrease in peroxidase activity with both time and temperature and the differences were most pronounced in the commercially prepared sample. Similar behaviour had been observed previously (5).

While no justification for cooling subsequent to drying should be drawn from peroxidase values they offer points of interest in continuing the investigation. It is possible that the poor palatability of the samples at 26.7° C. was the result of the action of peroxidase or other enzymes, and the poor palatability at 48.9° and 60.0° C. the result of temperature effects. The better quality of powders stored at 37.8° C. may then be the result of destruction of peroxidase or other enzymes at a rate sufficiently rapid to prevent the enzyme from causing any appreciable deterioration in the powder, while the temperature is still low enough to prevent marked heat deterioration effects. Further attention is being given to the role of enzymes in deterioration of milk powder quality.

Acknowledgments

References

1. AMERICAN DRY MILK INSTITUTE, INC., CHICAGO, ILL. The Grading of Dry Milk Powders. 1942.
2. BEATTY, S. A. and GIBBONS, N. E. J. Biol. Board Can. 3 : 77-91. 1937.
3. CHAPMAN, R. A. and McFARLANE, W. D. Can. J. Research, B, 21 : 133-139. 1943.
4. COE, M. R. Oil and Soap, 18 : 227-231. 1941.

5. DAHLE, C. D. and PALMER, L. S. J. Dairy Sci. 7 : 141-146. 1924.

6. DAVIS, W. B. Ind. Eng. Chem. (Anal. Ed.) 14 : 952-953. 1942.

7. DOOB, H., WILLMAN, A., and SHARP, P. F. Ind. Eng. Chem. 34 : 1460-1468. 1942.

8. EL-RAFEY, M. S. and RICHARDSON, G. A. J. Dairy Sci. 27 : 1-18; 19-31. 1944. '

9. GORTNER, R. A. Outlines of Biochemistry, 1st ed. John Wiley & Sons, Inc., New York. 1929.

10. GREENBANK, G. R. and HOLM, G. E. Ind. Eng. Chem. 17 : 625. 1925.

11. HOLLENDER, H. A. and TRACY, P. H. J. Dairy Sci. 25 : 249-274. 1942.

12. HOLMES, H. N. Laboratory Manual of Colloid Chemistry. 3rd ed. * John Wiley & Sons, Inc., New York. 1934.

13. HUNZIKER, O. F. Condensed Milk and Milk Powder. 4th ed. Pub. by the author, La Grange, Ill. 1926.

14. HURD, C. B. and LETTERON, H. A. J. Phys. Chem. 36 : 604-615. 1932.

15. JACK, E. L. J. Dairy Sci. 22 : 353-361. 1939.

16. LEA, C. H., MORAN, T., and SMITH, J. A. B. J. Dairy Research, 13 : 162-215. 1943.

17. MACK, E. and FRANCE, W. G. A Laboratory Manual of Elementary Physical Chemistry. 2nd ed. D. Van Nostrand Co., New York. 1934.

18. PEARCE, J. A. Can. J. Research, F, 22 : 87-94. 1944.

19. STEFFEN, A. H., HOPKINS, E. W., KLINE, R. W., and WHETZELL, G. H. U.S. Egg Poultry Mag. 49 : 308-310, 334-336. 1943.

20. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E. Can. J. Research, D, 21 : 1-7 1943.

21. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 194-202. 1943.

22. WOODCOCK, A. H. Can. J. Research, D, 21 : 90-97. 1943.

23. WOODCOCK, A. H. Can. J. Research, F, 23 : 117-122. 1945.

24. WOODCOCK, A. H. and TESSIER, H. Can. J. Research, A, 21 : 75-78. 1943.

SMOKED MEATS

IV. MEASUREMENT OF COLOUR AND COLOUR STABILITY OF WILTSHIRE BACON

By Jesse A. Pearce and A. H. Woodcock

SMOKED MEATS

IV. MEASUREMENT OF COLOUR AND COLOUR STABILITY OF WILTSHIRE BACON[1]

By Jesse A. Pearce[2] and A. H. Woodcock[3]

Abstract

Colour and colour stability of smoked and unsmoked Wiltshire bacon stored for periods up to 98 days at −18°, −9°, −1°, and 7° C. were measured by nine- and by three-filter methods. Differences in brightness between bacon sides were appreciable. Decrease in brightness with time was evident after 70 days at 7° C., but not until after 98 days at freezer temperatures. Smoking increased brightness.

There were marked differences in colour quality between bacon sides. Smoking decreased the proportion of red colour in the meat with corresponding increases in blue and green. Colour stability measurements on all samples showed that exposure caused a decrease in the proportion of red and an increase in green and green-blue.

Introduction

During the course of preliminary work on smoked meats it was noted that the colour of smoked meat was lighter and less stable on storage than that of unsmoked meat (3). A more comprehensive experiment was undertaken, primarily to assess the chemical changes occurring in smoked meat (2) but the opportunity was taken to record the colour changes as well. These were measured by two methods developed in these laboratories: the earlier method was based on the use of the three primary colour bands, red, green, and blue (4); the later method consisted of the more elaborate nine-filter method (5), designed to give more precise information.

Materials

It was known that bacon was of a highly variable nature. Therefore the experimental design permitted a comparison to be made between smoked and unsmoked portions of the same side of bacon, since smoking was the primary factor under consideration.

The material used was fully described elsewhere (2), and treatment may be outlined briefly as follows: the right and left sides of four hogs were cured, allowed to drain and partially mature for five days at 3.3°C. (38° F.), and the back and gammon removed from each side. After allotting two backs at random to each of the storage temperatures of −18°, −9°, −1°, and 7° C., (0°, 15°, 30°, and 45° F.) each back was divided into two approximately equal portions, one of which was smoked at an air temperature of approximately 60° C. (140° F.) for 14 hr.

Methods

Brightness (intensity) and colour quality measurements were made by a three-filter (4) and by a nine-filter method (5) used in this laboratory. In addition colour stability measurements (1) were made on samples exposed for 72 hr. at 7° C. and 95% relative humidity.

The nine-colour method (5) was designed primarily to measure colour quality, brightness of the sample relative to the standard white block being determined as an item separate from analysis of colour quality. By this method, chroma or colour quality was determined as light scattered by the sample within any one of the nine colour bands. It is expressed as a fraction of the total light scattered by the sample in relation to the light scattered by the standard white surface (magnesium carbonate). As has been previously pointed out (5), the analytical work can be reduced by selecting the most informative of the nine colour regions, appropriate to the experimental material.

The three-filter method (4) was designed and operated to distinguish a combination of brightness and colour quality in three regions of the spectrum (red, green, and blue), the colour value being determined from the scatter by the surface under investigation as a percentage of the colour scattered by the surface of the standard white block when both surfaces were illuminated by the same source. Brightness was considered to be the sum of the light scattered in the three colour regions.

Since it has been pointed out (5) that the colour quality data using the three-filter method were closely correlated with brightness data using the nine-filter method, an attempt was made to eliminate brightness effects in the three-filter method. This was done by taking the ratio of colour value by the three-filter method (Table III) to brightness by the nine-filter method.

Results

The small differences in the results of colour measurements on meat necessitated statistical treatment of the data (1, 3). For purposes of statistical analyses (2) results at 7° C. (chill temperature) could not be included with results at −1°, −9°, and −18° C. (freezer temperatures). Therefore results at chill temperature are shown by graphs of mean values at the chill temperature in relation to graphs of the average results for all freezer temperatures (Figs. 1 and 2).

The general lack of statistically significant differences that could be attributed to temperature effects, shown in Tables II and IV, is accounted for by the fact that differences between the sides of bacon were relatively enormous. There was no instance of parallel behaviour of any two sides at the same temperature. Although the statistical design permitted demonstration of significant storage effects, in most instances the inherent differences between sides were as great or greater than differences attributable to changes during storage. Thus, variability in the product was greater than any differences due to storage temperatures or times.
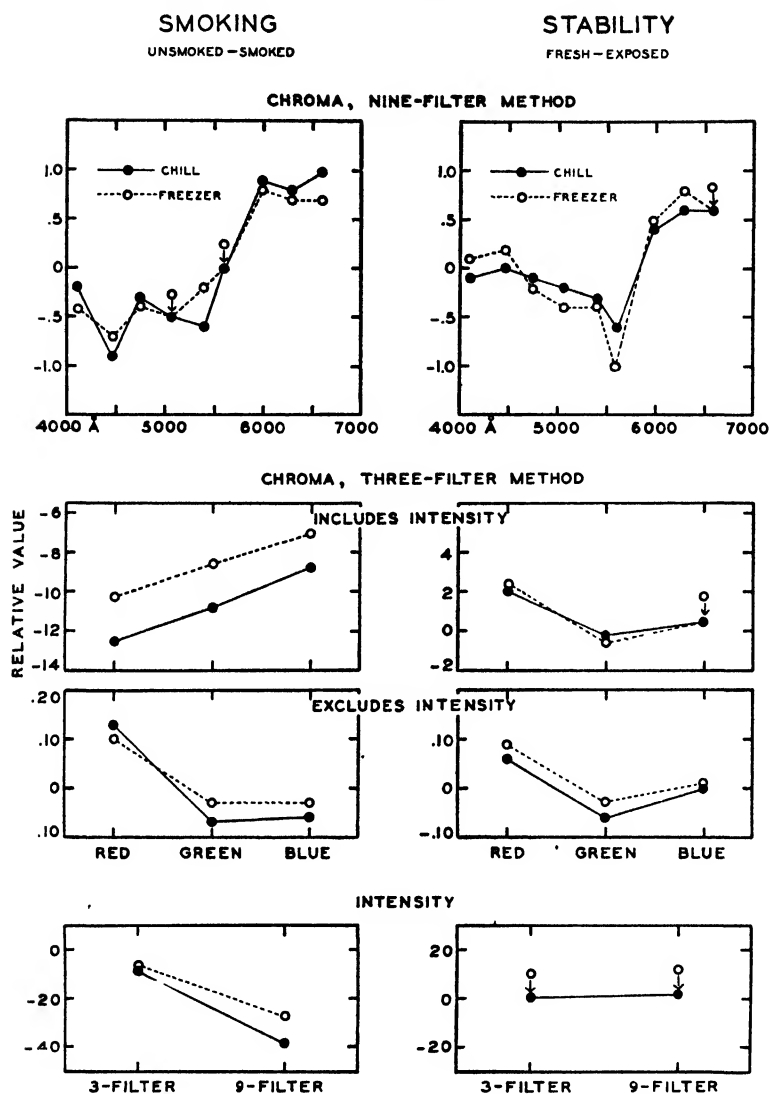
FIG. 1. *Relative differences resulting from the effect of smoking and stability tests on sides stored at chill and freezer temperatures.*

Brightness or Intensity

Measurements by the nine-filter method on samples stored at freezer temperatures showed that smoking increased and storage time decreased brightness, but exposure had no significant effect (Tables I and II). The only differential effect having practical significance was the result of a general increase in brightness of samples stored at $-18°$ and $-9°$ C. up to the 70-day sampling followed by a pronounced decrease at the 98-day sampling, while at $-1°$ C., a regular decrease occurred throughout the storage period. Smoked sides stored at the chill temperature had greater brightness than sides stored

CHANGES WITH TIME
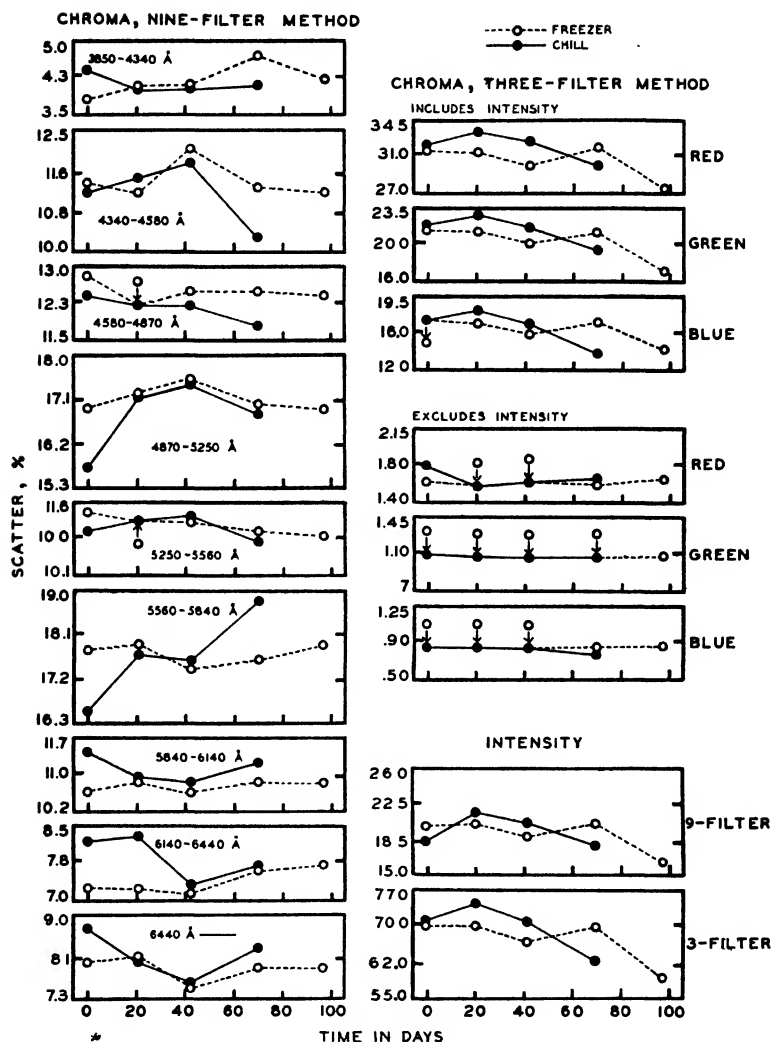
FIG. 2. *The effect of time on colour factors of meats stored at chill and freezer temperatures.*

at the other temperatures (Fig. 1), but brightness decrease with time was slightly greater (Fig. 2). Exposure again had only a minor effect on brightness.

Smoking, time, and exposure were observed to affect total brightness of samples held at freezer temperatures as measured by the three-filter method (Tables III and IV), the important difference from the nine-filter data being the significant decrease in brightness resulting from exposure. This might have been expected since the three-filter method gives an accumulative brightness effect, thereby amplifying any existing small differences. Smoking caused a greater increase of total brightness in sides stored at 7° C., and change on exposure was no different from that occurring in materials stored at freezer

TABLE I

Table of Means for Intensity and Colour Quality Measurements by the Nine-filter
Method on Smoked and Unsmoked Wiltshire Bacon Subjected to Storage
Temperatures of $-18°$, $-9°$, and $-1°$ C. and to a Stability Test at
$7°$ C. and 95% Relative Humidity

Variable under investigation	Inten-sity or bright-ness	Chroma or colour quality								
		Blue			Green			Red		
		3850–4340Å	4340–4580Å	4580–4870Å	4870–5250Å	5250–5560Å	5560–5840Å	5840–6140Å	6140–6440Å	Above 6440Å
Temperature (°C.)										
−18	18.7	4.3	11.2	12.4	17.1	11.1	17.8	10.7	7.4	8.0
− 9	19.7	4.0	11.5	12.5	17.2	11.2	17.8	10.7	7.3	7.8
− 1	19.1	4.2	11.'6	12.4	17.1	11.1	17.5	10.8	7.4	7.9
Smoking										
Unsmoked	15.3	4.0	11.1	12.2	16.9	11.0	17.7	11.1	7.7	8.2
Smoked	23.0	4.4	11.8	12.6	17.4	11.2	17.7	10.3	7.0	7.5
Time (days)										
Initial	20.0	3.8	11.4	12.8	16.9	11.4	17.8	10.6	7.2	8.0
21	20.3	4.1	11.2	12.2	17.2	11.3	17.9	10.8	7.2	8.1
42	19.0	4.1	12.1	12.5	17.5	11.2	17.4	10.6	7.1	7.5
70	20.2	4.7	11.3	12.5	17.0	11.0	17.6	10.8	7.6	7.9
98	16.4	4.2	11.2	12.4	16.9	10.9	17.9	10.8	7.7	7.9
Stability										
On removal from storage	19.3	4.2	11.5	12.3	16.9	11.0	17.2	11.0	7.8	8.2
After exposure	19.1	4.1	11.3	12.5	17.3	11.4	18.2	10.5	7.0	7.6

temperatures (Fig. 1). Decrease in brightness with time was evident after
70 days at $7°$ C. but not until the 98th day sampling at freezer temperatures
(Fig. 2).

Colour Quality—Nine-filter Method

Smoking, time of storage, and exposure all caused significant changes in
colour quality of samples stored at freezer temperatures (Tables I and II).
Smoking increased the proportion of blue and blue-green in the samples
and decreased the proportion of red. Differences in colour quality as storage
progressed were irregular and in general no marked change occurred. There
seems to be some evidence of a maximum or a minimum occurring at about the
42-day sampling (Fig. 2). Exposure after storage caused a general decrease
in the proportion of red and an increase in green and green-blue. Sides
stored at $-9°$ C. were much less susceptible to change on exposure.

The smoked sides used at the chill temperature had a greater proportion of
blue and a smaller proportion of red (Fig. 1). Changes in colour quality with
time (Fig. 2) again indicated a tendency for a maximum or a minimum to
occur at the 42-day sampling. Stability tests indicated that less drastic
colour changes occurred after chill storage (Fig. 1). This might again be

TABLE II

ANALYSIS OF VARIANCE OF INTENSITY AND COLOUR QUALITY MEASUREMENTS BY THE NINE-FILTER METHOD ON SMOKED AND UNSMOKED WILTSHIRE BACON SUBJECTED TO STORAGE TEMPERATURES OF $-18°$, $-9°$, AND $-1°$ C., AND TO A STABILITY TEST AT $7°$ C. AND 95% RELATIVE HUMIDITY

Source of variance	Degrees of dom	Intensity or brightness	Chroma or colour quality								
			Blue			Green			Red		
			3850–4340Å	4340–4580Å	4580–4870Å	4870–5250Å	5250–5560Å	5560–5840Å	5840–6140Å	6410–6440Å	Above 6440Å
Temperature	2	9 2	0 90*	2 1	0 12	0 06	0 20	1 4	0 22	0 29	0 44
Between sides within temperature (Error I)	3	135	0 38	1 4	0 32	2 1	0 88	2 5	0 86	1 0	4 8
Smoking	1	1785**	4 3**	14 0**	4 1**	6 9*	4 1**	0 00	18**	15**	17*
Smoking × temperature	2	89	0 37	2 9**	0 49*	0 79	0 32	0 06	0 29	0 73*	2 9
Smoking × sides within temp (Error II)	3	36	0 09	0 52	0 15	0 49	0 40	0 58	0 58	0 27	0 77
Time	4	65**	2 4**	3 1**	1 8**	1 6**	1 1**	1 2*	0 31	1 8**	1 3*
Time × temp	8	18*	0 21	0 35	0 13	0 60**	0 28	0 22	0 36*	0 74**	1 6**
Time × smoking	4	17	0 16	0 37	0 51**	0 05	0 05	1 0	0 26	0 44	0 56
Residual (Error III)	24	6 6	0 19	0 21	0 16	0 17	0 14	0 38	0 14	0 26	0 45
Stability	1	1 0	0 24	1 1*	1 5**	4 0**	5 3**	33**	7 4**	18**	11**
Stability × temp	2	0 16	0 -5	0 12	0 16	0 77**	0 64**	1 9**	0 20	1 3**	0 38**
Stability × smoking	1	0 03	0 32	0 24	0 01	0 18	0 04	0 38	0 11	0 07	0 00
Stability × time	4	0 81	*1 6**	0 30	0 63**	0 11	0 16	0 12	0 19	0 50	0 41
Residual (Error IV)	30	0 25	0 21	0 25	0 10	0 09	0 07	0 21	0 09	0 18	0 06
Machine variation	26		0 19	0 16	0 08	0 14	0 05	0 09	0 02	0 02	0 18

* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% level of statistical significance*

attributed to differences between sides, combined with the possibility that greater changes had occurred during storage at chill than occurred at freezer temperatures and therefore less change took place on exposure.

Colour Quality—Three-filter Method

The effect of smoking on samples stored at freezer temperatures appeared to be an increase in all three colours: this, however, was the result of the over-all increase in brightness since exclusion of brightness effects showed behaviour similar to that observed by the nine-filter method, i.e. a decrease in red and an increase in green and blue (Tables III and IV). This seemed to indicate that the proportion of red colour in smoked meats was smaller than in unsmoked meats although visually it appeared greater because of the brightness effects.

TABLE III

TABLE OF MEANS FOR INTENSITY AND COLOUR MEASUREMENTS BY THE THREE-FILTER METHOD
ON SMOKED AND UNSMOKED WILTSHIRE BACON SUBJECTED TO STORAGE TEMPERATURES
OF −18°, −9°, AND −1° C. AND TO A STABILITY TEST AT 7° C. AND 95%
RELATIVE HUMIDITY

| Variable under investigation | Total bright-ness | Chroma or colour quality | | | | | |
| | | Including intensity | | | Excluding intensity | | |
		Blue	Green	Red	Blue	Green	Red
Temperature (°C.)							
−18	65.5	15.8	20.0	29.7	0.85	1.06	1.61
− 9	67.8	16.3	20.6	31.0	0.84	1.04	1.59
− 1	66.8	16.0	20.0	30.2	0.84	1.04	1.60
Smoking							
Unsmoked	54.0	12.5	15.9	25.2	0.82	1.03	1.65
Smoked	79.6	19.5	24.5	35.4	0.85	1.06	1.55
Time (days)							
Initial	69.9	17.0	21.2	31 5	0.84	1.05	1.60
21	69.5	16.8	21.1	31.2	0.83	1.03	1.55
42	66.2	15.6	19.9	29.9	0.82	1.02	1.58
70	69.3	16.8	21.0	31.6	0.83	1.04	1.58
98	59.0	14.0	17.0	27.2	0.85	1.05	1.64
Stability							
On removal from storage	67.8	16.3	19.9	31.4	0 84	1.03	1.65
After exposure	65.5	15.8	20.5	29.1	0.83	1.06	1.54

Changes in colour quality resulting from time of storage at freezer temperatures corresponded closely with the changes shown to occur by brightness measurements. However, elimination of brightness effects indicated that in general storage time had little effect on the amount of any one of these three colours in the meat.

Colour quality measurements (including brightness) showed that decreases in red and blue and increases in green scatter resulted from exposure after storage. After excluding brightness only a decrease in red and increase in green was apparent. These latter results agreed with observations by the nine-filter method.

Colour quality values (excluding brightness) on samples stored at 7° C. showed changes attributable to smoking and exposure similar to those measured by the nine-filter method (Fig. 1). Time changes at the freezer temperatures measured by the three-filter method were largely attributable to changes in brightness; excluding brightness gave results similar to those occurring at the chill temperatures (Fig. 2).

Discussion

While the results by the nine-filter method have not been grouped as suggested (5) it is evident that the greatest number of significant changes

TABLE IV

ANALYSIS OF VARIANCE OF INTENSITY AND COLOUR QUALITY MEASUREMENTS BY THE THREE-
FILTER METHOD ON SMOKED AND UNSMOKED WILTSHIRE BACON SUBJECTED TO STORAGE
TEMPERATURES OF $-18°$, $-9°$, AND $-1°$ C., AND TO A STABILITY TEST AT
$7°$ C. AND 95% RELATIVE HUMIDITY

Source of variance	Degrees of freedom	Mean square						
		Total bright-ness	Chroma or colour quality					
			Including intensity			Excluding intensity		
			Blue	Green	Red	Blue	Green	Red
Temperature	2	59	3.5	4.5	16	0.0004	0.0078	0.0060
Between sides within temperature (Error I)	3	1369	101	163	201	0.0004	0.0002	0.0358
Smoking	1	19,630**	1478**	2205**	3154**	0.0323**	0.0163*	0.3070*
Smoking × temperature	2	1066	81	129	155	0.0016	0.0056	0.0280
Smoking × sides with temperature (Error II)	3	411	28	54	60	0.0012	0.0067	0.0119
Time	4	490**	39**	49*	83**	0 0032	0 0149*	0 0540**
Time × temperature	8	224*	18*	27	35	0.0017	0.0015	0.0045
Time × smoking	4	184	14	14	28	0 0002	0.0008	0.0038
Residual (Error III)	24	98	7.2	12	16	0.0021	0.0036	0.0057
Stability	1	158**	6.3**	11**	157**	0.0082	0.0347**	0.3532**
Stability × temperature	2	31**	1 3	0.45	20**	0.0028	0.0008	0.0638**
Stability × smoking	1	11	5 4**	1.0	9.9**	0.0078	0.0028	0.0006
Stability × time	4	4.7	1.4*	1 1	2.9*	0.0044	0.0012	0.0222**
Residual (Error IV)	30	4.1	0 50	1.5	0.80	0.0021	0.0029	0.0034

* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% level of statistical significance.*

occurred in bands 4580–4870Å and 6140–6440Å. The first of these bands is
considered to be a characteristic absorption band of nitrosohaemoglobin and
the second should represent changes in methaemoglobin (5). The results of
measurements in these two colour bands can be interpreted in terms of changes
in these components.

Temperature effects were not significant and time effects were extremely
variable. However smoking and exposure both appeared to increase the
nitrosohaemoglobin and to decrease the methaemoglobin. Interpretation of
these results, in terms of changes in components, must, however, be treated
with some reserve, since the method of making the measurement is dependent
upon the ratio of the amount of light scattered in the various bands. There-
fore, an increase in the light scattered in any one band must result in a decrease
of light scattered in one or more of the other bands.

With respect to the measurement of colour attributes of meat, the fore-
going indicates that the observations by either three- or nine-filter methods
were satisfactory. The three-filter method required adjustment of the colour
quality values to eliminate confusing colour quality with brightness. The
nine-filter method permitted a more precise differentiation of colour quality,
but in general the same conclusions can be drawn from results by either
method.

Acknowledgments

References

1. Cook, W. H., Gibbons, N. E., Winkler, C. A., and White, W. H.   Can. J. Research, D,
        18 : 123-134.   1940.
2. White, W. H.   Can. J. Research, F, 22 : 97-106   1944.
3. White, W. H., Gibbons, N. E., Woodcock, A. H., and Cook, W. H.   Can. J. Research,
        D, 20 : 263-275.   1942.
4. Winkler, C. A., Cook, W. H., and Rooke, E. A.   Can. J. Research, D, 18 : 435-441.
        1940.
5. Woodcock, A. H.   Can. J. Research, D, 21 : 90-97.   1943.

Statistical Estimation of Vitamin C Intake of Troops on Canadian Army Garrison Rations[1]

J. W. HOPKINS,[2] J. B. MARSHALL[3] AND J. C. CREASY[4]

THE intake of vitamin C by troops on army rations is determined by the ascorbic-acid content of the items served and their acceptability to the individual consumer. During the winter of 1941-42 two surveys were conducted to determine the individual daily intake of vitamin C from vegetable sources included in the regular issue of army garrison rations, by troops serving in the Base Post Office and the District Depot, M.D. 3, both in Ottawa. Estimates were deduced from chemical determinations of the vitamin C content of the vegetables, both fresh and cooked in various ways, and from statistics based on the amounts of vegetables actually consumed by individuals.

METHODS

Chemical

Representative samples of vegetables taken from each cooking-vessel at the time of serving were transported to the laboratory in $\frac{1}{2}$-pint sealers and extracted within 1 hour. Thirty-five grams of fresh or cooked vegetables were placed in a Waring blendor together with 150 ml. of 2 per cent metephosphoric acid to which 1 ml. of 1 per cent KCN had been added, and blended for 5 minutes under an atmosphere of carbon dioxide. The resulting suspension was poured into 250-cc. centrifuge bottles to which were added a further 30 ml. of acid used to wash the blendor jar, and the combined volumes centrifuged at 2000 r.p.m. for 15 minutes. (Washing was dispensed with in the later experiments.) Twenty-five ml. of the supernatant liquid were pipetted off and titrated with 2:6 dichlorophenol indophenol (80 mg. in 250 ml. water) for the determination of l-ascorbic acid. For the determination of dehydro-ascorbic acid, a further volume was reduced with hydrogen sulphide, the excess sulphide being removed with a current of nitrogen.

The moisture content of all samples was determined, and allowance made for the diluting effect on the volume of the extract in computing the analytical results.

In the tables which follow, the analytical results are expressed as milligrams of total ascorbic acid (reduced plus dehydro) per 100 grams of the material sampled.

Statistical

Statistical estimates of vegetable consumption by individuals were arrived at through a combination of enumeration and random sampling processes.

In both units the men were served in cafeteria fashion, and the number taking none, one or both of the vegetables available at any particular meal was determined by direct enumeration. All vegetable kettles were weighed immediately before and after serving, and the average weight of the individual portion of each vegetable computed. In order to determine the variability of individual servings, 15-20 representative servings issued by the Base Post Office cook were weighed immediately before each meal. The relative variance of seven series of successive helpings examined in this way was found to be of the same order, corresponding to a standard deviation of ± 13 per cent. In practice this basic variation in successive servings was increased as some individuals demanded more or less than the amount originally given. In consequence, a standard deviation of ± 20 per cent was considered to be more representative of the actual variability of individual servings and this figure was used in all subsequent calculations.

After each meal, the proportion of individuals leaving any residue of either or both vegetables uneaten was determined by enumeration of a representative sample comprising about 100 men in the case of the Base Post Office unit and 150-200 in the case of the District Depot. Individual weights of a random selection of 25-50 vegetable residues were also secured on each occasion.

From the foregoing enumerations it was possible to estimate for each meal the proportion of individuals in the consumption categories listed in Tables II and VI. These categories together with the preceding information with respect to the average weight and variation of servings and uneaten residues, and the ascorbic acid content of the vegetables as served provided the data necessary for estimation of individual vitamin C intake.

The range of intake of individuals taking one vegetable only but consuming the entire amount given to them may be specified by a frequency curve deducible from the mean weight of vegetable served, the variance of individual portions, and the ascorbic acid content of the material as served. In a similar manner, a frequency curve specifying the amounts of ascorbic acid left unconsumed by individuals taking one vegetable only and failing to eat their entire serving may be deduced. From the estimated parameters of these frequency curves it was possible to estimate the parameters of the frequency curve specifying the range of intake of individuals consuming only part of an initial serving of one vegetable. An extension of this process was used to deduce frequency curves of estimated vitamin intake deduced for individuals in each of the consumption categories specified. Then by reference to tables of the appropriate probability integrals the level of ascorbic acid intake bounding successive deciles of the area under each curve was determined and the scale of intake of the individuals in each category estimated, the upper limit being defined by the 99.9 per cent point of the curve, i.e., the ascorbic acid level having a theoretical probability of only 1 in 1000 of being attained or exceeded. Finally, the results for all categories were combined to yield an estimate of the total proportion of men whose intake of vitamin C from the meal in question was from 0 to 5, 5 to 10, 10 to 15 milligrams, etc.

RESULTS

Vitamin C Content of Raw and Cooked Vegetables

The results of chemical analyses of raw and cooked vegetables are summarized in Table I.

TABLE I

VITAMIN C CONTENT OF RAW AND COOKED VEGETABLES, EXPRESSED AS MG. TOTAL ASCORBIC
ACID PER 100 G. OF MATERIAL SAMPLED AT THE BASE POST OFFICE AND THE
DISTRICT DEPOT, M.D. 3, OTTAWA

Vegetable	Uncooked (sampled from stores)	As served		Percentage loss	
		Base P O	Dist Depot	Base P O	Dist Depot
Potatoes, boiled ..	12	3	3	75	75
" mashed	12	2	2	83	83
" fried or roasted	12	2	2	83	83
Cabbage, boiled	47	11		77	
" boiled + meat broth		3	5	94	89
Carrots, boiled	6	2	2	67	67
Coleslaw (cabbage, carrot, onion and vinegar)		36			
Peas, canned, boiled	11		5		55
" " boiled, liquor from	13				
Tomato, canned (served cold)	19	16	17	16	10
Turnips, boiled	44	7	1	84	98

In view of the time of year (February) at which observations were made, the vitamin C content of the samples taken from stores was commendably high in relation to the averages reported for fresh vegetables of the same species. It is also evident, however, that this originally high quality was largely dissipated by the cooking procedure then current and that the vitamin C content of all the cooked vegetables examined as served was low.

Vitamin C Intake of Base Post Office Troops

Observations were made in the Base Post Office mess for three days commencing February 10, 1942, attention being confined to the noon and evening meals in view of the negligible amount of vitamin C derivable from any of the breakfast items.* From the enumerations made on these occasions the statistics of consumption listed in Table II were deduced. The vegetables served

TABLE II

PERCENTAGE OF BASE POST OFFICE TROOPS IN VARIOUS CATEGORIES OF
VEGETABLE CONSUMPTION

Consumption category	February 10th		Feb 11*	Feb 12 †
	Dinner	Supper	Dinner	Dinner
Took no vegetable	1½	½		½
Took potatoes only, ate entire serving	15	20	28	27
Took potatoes only, left some uneaten	3½	4½	10	4
Took second vegetable only, ate entire serving	1½	3	1	½
Took second vegetable only left some uneaten	½			
Took both vegetables ate entire serving of both	36	48	14	25
Took both vegetables, ate all of second left some potatoes	15	12	16	8
Took both vegetables, ate all potatoes left some of second vegetable	11	2	6	17
Took both vegetables, left some of both	16	9	25	18
Took two servings of second vegetable		½		
Took two servings of potatoes				
Took two servings of both vegetables		½		
Number of men enumerated	192	178	201	184

*Supper—beef stew, statistics not enumerated, for reasons given
†Supper—roasted potatoes and cold canned tomatoes, statistics for February 10th used

*Since this survey was made, grapefruit juice, tomato juice and fortified apple juice have been included in the regular issue.

on each of these days, and the weight of average portions were as follows: February 10th, dinner: boiled potatoes (159 g.) and boiled carrots (109 g.); supper: roasted potatoes (148 g.) and cold canned tomatoes (76 g.); February 11th, dinner: boiled potatoes (140 g.) and boiled cabbage (88 g.); supper: beef stew with potatoes, cabbage and turnips; February 12th, dinner: mashed potatoes (292 g.), boiled potatoes (125 g.) and mashed turnips (129 g.); supper: roasted potatoes and cold canned tomatoes.

At dinner on February 10th, 36 per cent of the men ate an entire serving of both boiled potatoes and carrots; at supper 25 per cent took potatoes only, while 48 per cent of them consumed an entire serving of the two vegetables.

The boiled potatoes and cabbage offered at noon on February 11th were even less acceptable as only 14 per cent of those served were estimated to have eaten an entire serving of both vegetables. The vegetable constituents of supper on this date consisted of carrots and turnips unserved at previous meals and now included with potatoes in a beef stew. Detailed observations were not made for this meal as it can be assumed with confidence that the scale of vitamin C intake equivalent to that deduced for the day before would more than do justice to it.

Mashed potatoes served with turnips at dinner on February 12th appeared to be favoured by the men and were served more generously, the average serving being about double that for other forms of potato. At this rate of issuance the first 125 individuals exhausted the supply and the remainder of the 184 men enumerated received boiled potatoes. Again it was estimated that only 15 per cent of the men ate entire servings of both vegetables. As supper was a repetition of that served on February 10th, statistics for that day were applied again.

The average recorded weights of uneaten residues are shown in Table III. It will of course be realized that these figures imply not that, for example,

TABLE III

VEGETABLE RESIDUES LEFT UNEATEN BY BASE POST OFFICE TROOPS

Vegetable	Percentage of men		Average weight of serving, grams	Average weight of residue, grams	Average residue as per cent by weight of average serving
	Taking serving	Leaving residue			
Potatoes, boiled	96	43	159	64	40
Potatoes, roasted	96	26	148	54	36
Potatoes, mashed	99	30	292	46	16
Carrots, boiled	80	28	109	39	36
Cabbage boiled	62	31	88	21	24
Turnips, boiled	68	35	129	48	37
Tomato, cold	75	11	76	11	14

37 per cent of the entire quantity of turnips served was uneaten, but that it is estimated that this was the average proportion left by the 35 per cent of individuals who accepted a serving and then failed to consume all of it. A tendency of some individuals to leave the vegetables virtually untouched was most pronounced in the case of potatoes, but was evident in all instances.

By means of the computational procedure perviously outlined, the scale of

estimated individual vitamin C intake shown in Table IV was deduced for the four meals surveyed. From these estimates of intake at each meal separately,

TABLE IV

ESTIMATED VITAMIN C INTAKE OF BASE POST OFFICE TROOPS PER MEAL

Range of intake— milligrams	Percentage of individuals			
	February 10th		February 11th	February 12th
	Dinner	Supper	Dinner	Dinner
0 to 5	36	26	32	30
5 " 10	64	1	24	27
10 " 15		20	38	37
15 " 20		41	6	6
20 " 25		11		
Over 25		1		

further estimates may be made of the range of total individual intake from dinner and supper of each day, on the assumptions (*a*) that the choice of vegetables at supper by each man was statistically independent of his selection at dinner, and (*b*) that the scale of intake at supper on February 11th (beef stew and boiled potatoes) was equivalent to that at dinner on February 10th (boiled potatoes and carrots), the latter assumption being almost certainly over-optimistic. Thus for example in these circumstances, of the 26 per cent of individuals receiving an estimated 0 to 5 mg. from their consumption of vegetables at supper on February 10th, 26 × 36/100 or 9 per cent may be computed likewise to have had an estimated intake of 0 to 5 mg. at the preceding meal, and hence a possible combined intake of 0 to 10 mg. On the other hand 26 × 64/100 or 17 per cent had received an estimated 5 to 10 mg. at dinner and consequently had a possible combined intake of 5 to 15 mg. It will be noted that there is now some overlapping in the successive derived categories of possible intake, viz. 0 to 10, 5 to 15, 10 to 20 mg. etc.; but even if it be favourably assumed that none of the individuals in the class 5 to 15 mg. actually consumed less than 10 mg., that none of those in the class 15 to 25 mg. actually consumed less than 20 mg., etc., the estimated scale of daily intake would still be as indicated in Table V.

TABLE V

ESTIMATI) DAILY VITAMIN C INTAKE OF BASE POST OFFICE TROOPS FROM VEGETABLES EATEN

	Percentage of individuals			
	February 10th	February 11th	February 12th	Average
Less than 10	9	12	8	10
" " 20	34	70	30	45
" " 30	92	100	72	88
" " 40	99		98	99

These estimates may if anything still be somewhat optimistic by virtue of the fact that they assume, as explained above, a statistically independent choice of vegetables at dinner and supper of each day. In actuality it is likely that a certain proportion of the men had a definite distaste for any vegetable other than potatoes, and hence took potatoes only at both meals in somewhat greater numbers than calculated from chance probability. This would tend to increase the proportion of individuals in the lowest categories of vitamin intake. Moreover, as the remaining vegetables served during the week, viz. boiled

beets and baked beans, the former after cooking were of the same order as cabbage and turnips, and the latter were entirely negligible as sources of ascorbic acid, the indications were that this condition was chronic during the winter period.

Vitamin C Intake of District Depot Troops

Observations were made in the District Depot kitchen and mess hall on three successive days in February, when vegetables were served in the average amounts indicated: February 23rd, dinner: boiled potatoes (130 g.) and cabbage (80 g.); supper: potatoes (99 g.) and coleslaw containing cabbage, carrot, onion and vinegar (90 g.); February 24th, dinner: boiled potatoes (133 g.) and carrots (62 g.); supper: potatoes, some boiled, some fried (94 g.) and green peas, boiled (34 g.), served with roast beef sandwich; February 25th, dinner: boiled potatoes and turnips; supper: potatoes, some boiled, some fried (111 g.) and cold tomatoes (117 g.)

The deduced statistics of consumption of these are listed in Table VI. With

TABLE VI

PERCENTAGE OF DISTRICT DEPOT TROOPS IN VARIOUS CATEGORIES OF VEGETABLE CONSUMPTION

Consumption category	February 23rd		February 24th		February 25th	
	Dinner	Supper	Dinner	Supper	Dinner	Supper
Took no vegetable	¼	2½	¼	½	1	¼
Took potatoes only, ate entire serving	13	11	12½	½	15½	13
Took potatoes only, left some uneaten	2	4	2½	¼	2	3½
Took second vegetable only, ate entire serving	1½	1½	½	6	½	¼
Took second vegetable only, left some uneaten	¼	½			¼	
Took both vegetables, ate entire serving of both	50	31	34	58½	30	45½
Took both vegetables, ate all of second, left some potatoes	11	20	13	20	9	16
Took both vegetables, ate all potatoes, left some of second vegetable	10	13	11	7	17	6
Took both vegetables, left some of both	12	16	26	6	24	15
Took two servings of second vegetable		½		¼	¼	¼
Took two servings of potatoes				¼	¼	
Took two servings of both vegetables				¼		
Took one serving of potatoes, two of second vegetable						¼
Number of men enumerated	685	496	724	558	750	536

one exception (supper on February 24th, when the meat courses consisted of a roast beef sandwich), the indicated habits of vegetable consumption were similar to those prevailing in the Base P.O. unit, 15 to 20 per cent of the men taking potatoes only (as compared however to a maximum of 30 per cent in the B.P.O.) and not more than 50 per cent eating an entire serving of both vegetables at any given meal. The average recorded weights of uneaten residues are listed in Table VII. From these, it would appear that tomatoes were the most palatable and turnips the least palatable of the seven vegetables examined.

Using the same method of computation as before, the scale of individual vitamin C intake listed in Table VIII was arrived at for each meal. The contrast in intake from cooked and uncooked cabbage (coleslaw) is notable. Both carrots and peas are relatively poor sources of vitamin C. Actually the peas as served had a content 2½ times that of the carrots, but this was offset by the fact that the average portion of the former weighed only 34 g. Whilst

TABLE VII

VEGETABLE RESIDUES LEFT UNEATEN BY DISTRICT DEPOT TROOPS

Vegetable	Percentage of men		Average weight of serving, grams	Average weight of residue grams	Average residue as per cent by weight of average serving
	Taking serving	Leaving residue			
Potatoes	95	34	116	49	42
Cabbage	84	22	80	32	40
Carrots	84	37	62	25	40
Coleslaw	82	29	90	39	43
Peas	98	14	34	12	35
Tomatoes	84	21	117	32	27
Turnips	81	41	62	35	56

TABLE VIII

ESTIMATED VITAMIN C INTAKE OF DISTRICT DEPOT TROOPS PER MEAL

Range of intake, milligrams	Percentage of individuals					
	February 23rd		February 24th		February 25th	
	Dinner	Supper	Dinner	Supper	Dinner	Supper
0 to 5	26	20	69	99½	85	17
5 " 10	71	3	31	½	15	3
10 " 15	3	3				7
15 " 20		6				26
20 " 25		10				34
25 " 30		12				10
30 " 35		17				2
35 " 40		15				1
Over 40		14				

notably superior to that from any of the cooked vegetables, the intake from tomatoes was not equal to that from coleslaw.

The scale of individual daily intake was deduced to be as shown in Table IX. The superiority of coleslaw as a source of vitamin C is clearly reflected

TABLE IX

ESTIMATED DAILY VITAMIN C INTAKE OF DISTRICT DEPOT TROOPS FROM VEGETABLES EATEN

Daily intake milligrams	Percentage of individuals			
	February 23rd	February 24th	February 25th	Average
less than 10	5	69	14	29
" 20	24	100	26	50
" 30	34		82	72
" 40	57		99	83
Over 40	43		1	15

in the results for February 23rd, on which day it seems likely that about half the men had an intake of at least 40 mg. Even on this occasion, however, it is estimated that one-quarter of the men received less than 20 mg., and in view of the much less favourable results deduced for February 24th and 25th, together with the fact that the only other vegetable appearing on the diet sheet at this time, namely boiled beets, is inferior to tomatoes in ascorbic acid content, it must be concluded that on the average at least 70 per cent of the men were in receipt of less than 30 mg. daily intake from vegetable sources.

Effect of Conservation of Vitamin C on Estimated Intake

It is possible by means of improved cooking methods to conserve more of the vitamin C content of the vegetables served. This would have a marked effect on the level of daily intake of the men. It could not, however, be expected to eliminate completely the deficiencies indicated in the preceding

section as will be appreciated when it is recollected that at each meal surveyed, 15 to 30 per cent of the men ate potatoes only or no vegetable at all.

As a matter of interest, the estimated range of daily intake shown in Table X was computed on the basis of the observed amounts of vegetables actually

TABLE X

ESTIMATED INTAKE OF TROOPS, ASSUMING 50 PER CENT RETENTION OF ORIGINAL VITAMIN C CONTENT OF VEGETABLES

Daily intake, milligrams	Percentage of individuals			
	Base Post Office			Base Depot
	February 11th	February 12th	Dinner, Feb. 11th Supper, Feb. 12th	February 23rd
Less than 10.............	1	2	1	1
" " 20.............	36	16	12	5
" " 30.............	59	55	41	10
" " 40.............	94	84	67	26

consumed on the days indicated, but assuming that 50 per cent of the original vitamin content was retained in the material served. Had the cabbage and potatoes served at the Base Post Office on February 11th occurred in conjunction with an evening meal including cold tomatoes and potatoes, as on February 12th, the computed range of intake would have been as shown in column four of the table, and 40 per cent of the men still would have been in receipt of less than 30 mg.

At the Base Depot on February 23rd, 26 per cent of the men would have received less than 40 mg. This, however, represents the range of intake that might be expected when the two richest sources of vitamin C in the available dietary were served on the same day, i.e. boiled cabbage at dinner and coleslaw at supper. The results for other vegetables would approximate more closely those computed for the Base P.O. unit on February 11th and 12th.

In view of the observed dietary habits and in the absence of sources other than the vegetables served, an appreciable number of individuals could be expected to have a daily intake of less than 40 milligrams of vitamin C.

SUMMARY

Analytical and statistical data obtained from surveys conducted during the winter of 1941-42 have been used to estimate the individual daily intake of vitamin C from vegetable sources included in the regular issue of army garrison rations, by troops of two units in Ottawa. Current methods of cooking left only a small fraction of the original vitamin C concentration in the food as served.

Of the troops served, 15-30 per cent refused any vegetable other than potatoes and about 1 per cent took no vegetable. In some instances, as high as 50 per cent of the men failed to eat the entire serving they received; the uneaten residues averaged from 15 per cent by weight of the average original serving for tomatoes to 56 per cent for turnips.

From the foregoing facts it was estimated that about 88 per cent of the men in one unit and 70 per cent of those in the other had a daily vitamin C intake of less than 30 mgs. from vegetable sources. Increased retention of vitamin C in cooking will not overcome deficiencies arising from consumption habits.

CALIBRATION AND CHARACTERISTICS OF A SENSITIVE HOT-WIRE ANEMOMETER

BY T. A. STEEVES, A. E. CHADDERTON AND W. H. COOK

CALIBRATION AND CHARACTERISTICS OF A SENSITIVE HOT-WIRE ANEMOMETER[1]

By T. A. Steeves[2], A. E. Chadderton[3] and W. H. Cook[4]

Abstract

A wet test meter was used to measure the air supplied to a small wind tunnel that could be adjusted to provide up-, down-, or side-draughts for the calibration of a sensitive hot-wire anemometer. The readings on the instrument used were independent of the direction of air flow and the orientation of the instrument at velocities in excess of 10 ft. per min. At lower velocities separate curves were obtained for different instrument orientations and directions of air movement. Air flows as low as 1 ft. per min. can be estimated with useful accuracy provided the direction of air movement is known.

Introduction

Air movement in cold storage rooms is an important factor in relation to spatial temperature variations and the rate of evaporation from stored goods. Within a stack of perishable products the air movement is generally less than 10 ft. per min. and may occur in almost any direction. As methods based on smoke movement are generally unsatisfactory and undesirable in stacks of edible goods, attention was turned to the use of a hot-wire anemometer.

In the course of these studies it was found that while instruments of this type could readily be standardized at zero air velocity when the sensitive portion was covered with a close fitting cover, readings as high as 5 ft. per min. were observed when the cover was removed. Obviously the air in a closed laboratory is not "still" even when precautions are taken to avoid draughts. It was therefore evident that the rotating arm method (2) was unsuitable for calibrating a sensitive instrument.

Most instruments of this type show some variation in the reading when rotated or orientated in different positions in a slow moving air stream (5). The extent of these variations depends on the air velocity and design of the instrument. Thomas (6) describes a directional heat transfer anemometer that lost its directional sensitivity at gas velocities in excess of 20 ft. per min., even at wire temperatures of 500 to 800° C. Instruments employing lower wire temperatures might reasonably become independent of directional effects at lower velocities.

At the air velocities of interest in the present application, instruments having the necessary sensitivity usually behaved normally in "up-draughts" but showed negative values in low velocity "down-draughts" such as those

[2] *Refrigerating Engineer, Food Investigations.*
[3] *Senior Laboratory Assistant, Food Investigations.*
[4] *Director, Division of Applied Biology.*

produced by a cooling coil.   Having secured a sufficiently sensitive instrument, methods had to be devised for its calibration in relation to the orientation of the instrument and the direction of air movement.

Description of Instrument

In addition to sensitivity, a satisfactory instrument for the duties indicated must be readily portable, capable of being inserted into spaces in stacked products, and independent of temperature effects.   These requirements appeared to be met in a commercial instrument devised by Albrecht (1, pp. 390-397) and manufactured by R. Fuess, Berlin, Germany.   Since the reported results were obtained from this instrument it will be described briefly.
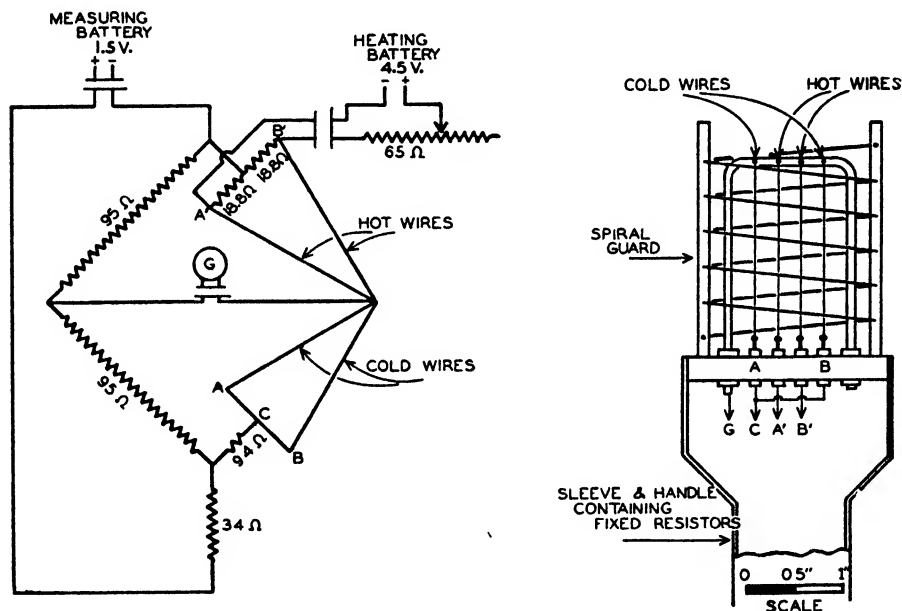
FIG. 1.   Circuit and arrangement of hot and cold wires in Albrecht anemometer.

A drawing of the sensitive portion of the instrument and the circuit employed is shown in Fig. 1.   Two sets of dry cells are required, one for heating the "hot" wires and the other for the measuring circuit.   The hot and cold wires were originally made from 0.0006 in. diameter platinum, although 0 001 in. wires were later substituted, with no apparent effect on sensitivity. The use of platinum wires in both the "hot" and "cold" wire arms of the bridge compensated the instrument for changes in ambient temperature. The sensitive portion is provided with an extension handle containing the coils and extension leads to the galvanometer, batteries, and adjustable resistor.   The galvanometer had two scales, one linear electrical scale (0.6 μa. per scale division) and the other an air movement scale, reading from right to left and graduated at approximately, but not exactly, logarithmic intervals.

The sensitive portion of the instrument was provided with a heavy metal cover to be used for protection when not in use and for standardization. The instruction called for the standardization to be done with the cover in place and the instrument in a vertical position, sensitive end down. In this position the heating current was adjusted by means of the resistance to bring the galvanometer to zero on the air flow scale or practically full scale electrical deflection.

The initial experiments showed that the instrument could be standardized in almost any position, although the stability of the zero adjustment was somewhat better when the recommendations were followed. Even with the cover in place, the instrument was sensitive to position; otherwise it was quite stable and returned to the zero value when returned to the vertical axis. Since air movement other than that induced by the "hot" wires must have been negligible, these observations indicated that the instrument was sensitive to orientation and this factor had to be taken into account in making the calibration.

Method of Calibration

Ower (4) describes a modification of the rotating arm method using an annular tunnel to avoid stray air currents but it was felt this would not readily lend itself to various instrument orientations. Other investigators (7) report difficulties with the rotating arm which introduces variables of too great magnitude for low velocity calibration. The present method of calibration was to deliver air at a constant rate through a precision gas meter to a small draught tunnel of known cross-section.
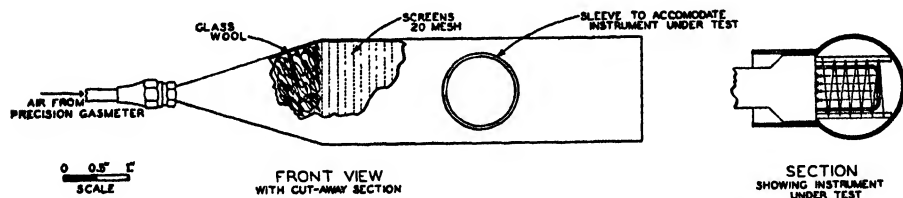
FIG. 2. *Draught-tunnel for calibrating hot-wire anemometer.*

This apparatus is shown diagrammatically in Fig. 2. A compressed air line fitted with pressure regulating devices and restrictions ahead of the gas meter insured uniform rates of flow within narrow limits. The flow rates were also checked by timing the movement of the indicating needles on the gas meter. Even minor fluctuations in flow rate were detected by the anemometer, and the observations were not recorded unless a steady condition prevailed.

Uniform air distribution throughout the tunnel was insured by filling the funnel-shaped entrance with glass wool followed by 10 layers of fine mesh screen in the cylindrical section. The tunnel exit was also covered with screen to eliminate the effect of local air currents. The side arm of the tunnel was arranged to take the instrument in any orientation. Since the tunnel itself

could be mounted in any position the air motion could be adjusted to represent side-, up-, or down-draughts.   In this way almost any conceivable combination of directions of air motion or instrument orientation could be tested.

Visible examination using smokes showed that distribution of air movement within the tunnel was excellent.   With the instrument and tunnel in any given position it was found that the results were remarkably reproducible from time to time.

Results

A number of calibration curves corresponding to different instrument orientations and directions of air movement were obtained, but only six curves, representing the combinations of greatest interest and practical importance, are reproduced in Fig. 3.   These show the velocity in feet per minute plotted against the linear electrical scale divisions.   In all instances the instrument was standardized at 80 scale divisions outside the tunnel with the protecting cover in place and the instrument in a vertical position.
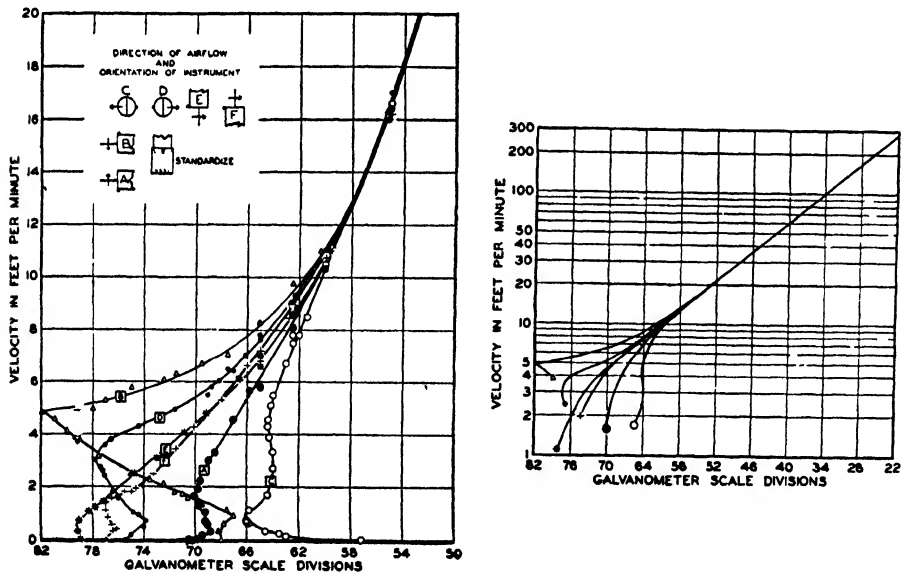
FIG. 3.   *Calibration curves for various instrument orientations and directions of air flow.*

Following standardization the cover was removed and the instrument placed in the tunnel.   The reading obtained at zero air velocity was dependent on the position of the instrument but was always less than the standardization value of 80.   With the wires in one of the arrangements (Position *C*) the reading at zero air velocity was less than 60 scale divisions, a value corresponding to an air velocity of about 10 ft. per min. on most of the curves. These effects are attributable to the orientation of the instrument causing either an excessive cooling of the "hot" wires or excessive heating of the "cold" wires in the opposite arm of the bridge.   The latter would appear to

be the more logical explanation.   The difference between Positions C and D must be attributed to different resistance and temperature effects in the two parallel hot or cold wires.   This explanation was confirmed in part by changing the wires in the instrument.

At low air velocities the readings may either increase or decrease, depending on the position under test.   All the curves show sharp changes of direction at velocities between 0 and 1 ft. per min.   This behaviour indicates interacting effects between instrument orientation and air velocity.   The position and form of a particular curve are still determined primarily by the orientation of the instrument, and the initial reading at zero velocity, rather than by the rate of air movement.   While the individual curves were reproducible the instrument is obviously of little value for estimating air velocities less than 1 ft. per min.

At velocities above 1 ft. per min. smooth curves were obtained when the instrument was placed vertically in a horizontal air flow.   Curves representing other conditions, in particular the one obtained with a down-draught, show directional changes at velocities below 5 ft. per min.   The curve for a down-draught (Position B) shows that the readings increase rather than decrease with increasing air velocity between 1 and 5 ft. per min. and then reverse sharply.   This indicates that the effective upward air velocity induced by the "hot" wires corresponds to about 5 ft. per min., a figure in agreement with the value computed by King (3).   Down-draughts of lower velocity reduce this convection, and cooling of the hot wires is therefore retarded.

At velocities above 5 ft. per min. all the curves were smooth although they differ in position at air speeds less than 10 ft. per min.   Curves representing all orientations and air directions tested coincide at velocities above this value, and yield a straight line when plotted on semilog paper.

Discussion

While some attempt has been made to explain the observed behaviour of the instrument, a detailed study of these points was not undertaken.   The influence of instrument orientation is doubtless peculiar to the particular design and instrument.   The differential effect of up-, down-, and side-draughts may reasonably be common to all instruments of this type.

The instrument cannot be considered of any value for measuring air velocities less than 1 ft. per min.   In the special case of a horizontal air stream in which the instrument can be placed in a vertical position, useful estimates can be made at velocities in excess of 1 ft. per min.   In practice, low velocities in a truly horizontal direction are not likely to occur to any great extent.   The more common low velocity air currents are those in which the vertical component predominates; when the velocity of such currents is less than 5 ft. per min., the present instrument is of little value unless the direction of air movement is known and the orientation of the instrument standardized. Similar precautions are necessary for measuring velocities between 5 and 10

ft. per min. but in this range estimates of varying accuracy can be made without knowing the precise direction of air movement. At velocities in excess of 10 ft. per min. the instrument is independent of directional components.

References

1. ALBRECHT, F. Handbuch der meteorologischen Instrumente und ihrer Auswertung. Springer, Berlin. 1935.
2. KING, L. V. Trans. Roy. Soc. (London) A, 214 : 373-432. 1914.
3. KING, L. V. Phil. Mag. Ser. 6. 29 : 556-577. 1915.
4. OWER, E. The measurement of air flow. Chapman & Hall, London. 1933.
5. SIMMONS, L. F. G. and BAILEY, A. Aeronautical Research Committee Reports and Memoranda No. 1019. H.M. Stationery Office, London. 1926.
6. THOMAS, J. S. G. Phil. Mag. Ser. 6. 41 : 240-258. 1921.
7. VERNON, H. M. and MANLEY, J. J. Medical Research Council Special Report Series No. 100. H.M. Stationery Office, London. 1923.

Water, Microorganisms and Volatile Bases in Dehydrated Fish

By H. L. A. Tarr

Pacific Fisheries Experimental Station

ABSTRACT

The relations of relative humidity (R.H.) to water content in precooked, air-dehydrated fish were determined with reference to the mould, bacterial and volatile-base content of stored samples. In general it was found that visible mould developed only in samples stored at 80% or higher R.H., while bacteria increased only at 85% or higher R.H. Viable bacteria and moulds decreased slowly in samples stored at R.H. too low to support their active multiplication. Total volatile bases and trimethylamine increased in samples stored at R. H. too low for growth of either bacteria or moulds.

Artificial dehydration of fish is by no means a recent innovation, for as long ago as 1877 and 1880 patents were issued to Alden for methods of preparing precooked dehydrated fish (cited by Stevenson 1899). The possibility of utilizing dehydrated fish as a convenient economical source of animal protein in human nutrition was recognized during the war of 1914 to 1918, for about that time methods of preparing both raw and precooked dehydrated fish were described (Duge 1913, cited by Tressler 1923; Falk, Frankel and McKee 1919). It is not clear whether any of the processes described were applied commercially. During the present emergency renewed interest has been taken in this type of product. Cutting and Reay (1944), using equipment previously described by Cutting (1942), showed that precooked dehydrated fish was easier to produce than raw dehydrated fish, though Kidd (British Patent 539,477 in 1938) had previously produced an apparently satisfactory raw dehydrated fish by drying the minced frozen flesh under fairly high vacuum ("Vac-ice" process). In general, most workers appear to agree that the best practical results are obtained by pre-cooking whole gutted fish in steam, flaking the flesh from the bones (the cooked flesh may be pressed to remove some of the fat and excess watery liquid), mincing, and drying in a stream of warm air (Young and Sidaway 1943; Young and Lee 1943; Martinek and Jacobs 1943; Cutting and Reay 1944). The equipment and individual methods used by different investigators have varied, but it appears to be generally agreed that dehydration of the minced cooked flesh should be completed in a few hours and that the temperature of the product should not rise above 60 or 70°C. during processing. The present investigation was undertaken with a view to determining some of the conditions necessary for satisfactory storage of precooked dehydrated fish; a preliminary report has already been published (Tarr 1943).

EXPERIMENTAL

Only fresh fish were used. These were precooked and, with one exception, dehydrated by the process developed at this Station (Young and Sidaway 1943). In general a temperature range of 50 to 60°C. was employed in the dehydration, care being taken not to overheat the fish. It was assumed that minor variations in temperature and time employed in dehydration did ñot affect the results of the experiments to be described. ·Viable bacteria and mould and yeast counts were made by the roll tube method (Tarr and Bailey 1939), the dehydrated fish samples being ground mechanically with sand for 5 minutes with fourteen times their weight of sterile water prior to analysis. Double strength Bacto nutrient agar containing 0.02 M phosphate buffer (pH 7) was employed for the bacterial counts. The same medium containing added glucose and tartaric acid in the proportions described by Henrici (1930) was usually employed for mould and yeast counts. In general, the ordinary medium used favoured growth of bacteria, while the glucose-tartaric acid medium favoured growth of moulds and yeasts. However, both yeasts and moulds occasionally developed on the former, and yeasts, and possibly certain bacteria, on the latter, so that the differentiation was not very sharp. In the experiments to be described all colonies, except obvious moulds, which developed on the ordinary medium were recorded as "viable bacteria," while all those which developed on glucose-tartaric acid medium were recorded as "moulds," though with certain samples numerous yeasts developed on this medium. In some instances where large numbers of mould colonies grew on the ordinary medium they were counted and the results recorded separately. Total volatile base (probably largely ammonia plus trimethylamine) and trimethylamine determinations were made on aqueous extracts, previously prepared for determining the number of microorganisms, using the Conway dish technique (Tarr and Bailey 1939). This method, which employs formaldehyde to bind ammonia and monomethylamine, determines trimethylamine and dimethylamine, so that the figures given for trimethylamine in this paper include any dimethylamine which may have been present in the samples. Constant humidity experiments were carried out in small glass desiccators, or similar vessels, containing 200 ml. of a solution of either calcium chloride (Macara 1943) or sulphuric acid of a concentration calculated to maintain the desired relative humidity (R.H.) at 25°C. Samples of dehydrated fish weighing about 10 g. were placed in 9-cm. sterilized open petri dishes on glass tripods over these solutions, the lids of the containers being sealed with a heavy grease made of vaseline and paraffin wax prior to storing them.

Water content was determined on approximately 2-g. samples of dehydrated fish by exposing them to a temperature of 100 to 101°C. for 24 hours in an air oven, cooling in the presence of phosphorus pentoxide, and weighing. In this connection it is of interest to note that moisture has been determined in dehydrated meat with apparently satisfactory results by distillation with toluene (Bate-Smith, Lea and Sharp 1943), and by drying to constant weight over phosphorus pentoxide at 30°C. or by drying for 6 hours at 100°C. in an air oven (Gane 1943). Drying in partial vacuum at 100°C. to constant weight has also been recommended (A.O.A.C. Official Methods of Analysis 1925). In the writer's experience samples of dehydrated fish do not attain absolutely constant

weight after 24 hr. at 100°C. in an air oven, but continue to lose a small amount of weight very slowly for many days when kept at 100°C. They simultaneously become very dark in colour.

ALTERATIONS IN MICROBIOLOGICAL CONTENT

Determinations of the numbers of bacteria and of yeasts and moulds per gram were made on samples of herring (*Clupea pallasii*), lingcod (*Ophiodon elongatus*), coho salmon (*Oncorhynchus kisutch*) and chum salmon (*Oncorhynchus keta*) at different stages in the dehydration process, namely (a) whole fish after precooking, (b) minced fish, and (c) dehydrated fish. The bacteria were as follows: herring,—(a) 14, (b) 720, (c) 17,000; lingcod,—(b) 96, (c) 930; coho salmon,—(b) 820, (c) 4,700; chum salmon,—(a) 0, (b) 79 and 390, (c) 23,000 and 1,800. The moulds were: lingcod,—(b) 8, (c) 22; coho salmon,—(b) 8, (c) 60; chum salmon,—(a) 0, (b) 3 and 8, (c) 380 and 45. There was thus a progressive increase in bacteria and moulds during mincing and dehydration of the cooked flesh. Factors such as contamination during mincing, concentration of organisms due to shrinkage of the material during dehydration, aerial contamination and, possibly, growth of organisms during the early stages of dehydration, were probably responsible for this.

Nineteen colonies which developed on ordinary medium in roll tubes from seven different samples of dehydrated fish, some freshly prepared and some after storage for three months, were examined microscopically. Of these, sixteen were cocci or very short fat rods almost coccoid in shape, two were yeasts and one was a spore-forming rod. Small gram-negative rods of the *Achromobacter* or *Pseudomonas* type were not found.

LINGCOD AND COHO SALMON STORED OVER CaCl₂

The sample of dehydrated lingcod used differed from the other samples of dehydrated fish employed in this work in that the minced cooked flesh was dried in a rather thick layer in a very slow stream of air for about a day at 45 to 50°C. These conditions favoured bacterial growth as is shown by the high initial bacterial content (table I), while the dried product was hard and did not possess the crispness of samples prepared in the usual manner. The dehydrated lingcod contained 77%, and the coho salmon 83% protein. Samples were stored at 25°C. over calcium chloride solutions at varied R.H.; the development of visible mould was recorded and viable bacterial counts and water determinations were made at stated intervals. It will be seen from table II that visible mould developed in samples stored only at 80% or higher R.H., corresponding to a water content of 17.2% and 17.9% of coho and lingcod respectively. Bacteria increased very rapidly at 90 and 100% R.H. At 85% R.H., af er 18 days there was an apparent increase beyond the initial bacterial content of the coho salmon, whereas after the same period there was a marked decrease from the initial high bacterial content of the lingcod. Viable bacteria decreased rapidly at first (as between initial values and those after 15 days' storage) and then more slowly (as between 45 and 112 days) in samples stored at from 65 to 80% R.H. In the sample of coho salmon stored for 112 days at 75% R.H. a mould count much higher than that found in certain samples of freshly prepared dehydrated

TABLE I. Effect of storing over calcium chloride solutions at different relative humidities on mould development, water and bacterial content of dehydrated lingcod and coho salmon.

	Species of fish	Initial values	Relative humidity (%)						
			65	70	75	80	85	90	100
Time before appearance of mould (days).	Coho salmon		*	*	*	34**	18	9	7
	Lingcod		*	*	*	52**	18	9	7
Water content at time of first appearance of mould (%). In samples in which visible mould did not develop, the water content was determined after 45 days' storage.	Coho salmon	4.8	11.8	12.8	13.5	17.2	19.2	27.6	29.4 (37.5)†
	Lingcod	2.6	12.2	13.1	14.1	17.9	19.7	30	28.3 (37.3)†
Water content after 112 days' storage (%).	Coho salmon	4.8	11.5	12.4	13.5	16.6			
	Lingcod	2.6	12.4	13.0	14.3	18.3			
Viable bacteria at first appearance of mould (colonies per g.). In samples in which visible mould did not develop, this determination was made after 45 days' storage.	Coho salmon	13,000	53	140	410	120	86,000	180×10^6	440×10^6
	Lingcod	16×10^6	110,000	190,000	37,000	240,000	1.5×10^6	250×10^6	370×10^6
Viable bacteria after 112 days' storage (colonies per g.)	Coho salmon	13,000	38	15	0	0			
	Lingcod	16×10^6	25,000	41,000	19,000	0			.
Viable moulds after 112 days' storage (colonies per g.). These developed and were counted on the medium used for determining numbers of viable bacteria	Coho salmon		300	75	1,500	4,700			
	Lingcod		0	0	0	2,600			

*No visible mould developed after 112 days' storage.
**Only one small patch of mould developed.
†The values given in brackets were obtained after 12 days' storage when the samples were extremely mouldy.

fish was found. Unfortunately initial mould counts were not made on the freshly dehydrated fish so that there was no definite means of knowing whether the observed rather high count was due to actual growth of mould, though the results of the next experiment indicated that some mould growth occurs at this humidity.

CHUM SALMON STORED OVER CaCl₂

This experiment was carried out under conditions similar to those described in the foregoing one with the exception that total volatile base and trimethylamine determinations were made. The results are given in table II. As far as the relation between R.H., water, bacterial content and visible growth of mould were concerned the results were, in general, closely similar to those obtained in

TABLE II. Effect of storing over calcium chloride solutions at different relative humidities on mould development and water, bacterial and volatile base content of dehydrated chum salmon. A is value obtained either at first appearance of mould or, in those samples in which visible mould did not develop, after 35 days' storage; B is value obtained after 102 days' storage.

		Initial values	Relative humidity						
			65	70	75	80	85	90	100
Time before first appearance of mould (days)			*	*	*	35 †	17	8	6
Water content (%)	A	4.1	11.0	12.1	13.6	16.8	19.0	23.2	33.1
	B	4.1	10.8	11.9	13.2	16.7			
Viable bacteria (colonies per g.)	A	23,000	5,300	12,000	15,000	61,000	1,100	7.6×10^6	67×10^6
	B	23,000	225	500,000	150	570			
Viable moulds developing on the medium used for bacterial counts (colonies per g.)	A	380	0	0	0	120,000	51,000	2.6×10^6	140×10^6
	B	380	165	0	64,000	>100,000			
Total volatile base (mg. N per 100 g.)	A	38	66	77	53	46	22	9.1	37
	B	38	82	81	46	26			
Trimethylamine (mg. N per 100 g.)	A	0	12	12	8.5	8.9	4.2	1.9	5.3
	B	0	27	33	17	22			

*No visible mould developed in 102 days.
†Only a moderate growth of mould occurred and this sample was stored for 102 days.

the foregoing experiment. The rather low bacterial count obtained in the sample stored for 17 days at 85% R.H., and the exceptionally high bacterial count obtained in the sample stored for 102 days at 70% R.H. are difficult to explain, though it is not unlikely that sampling errors were responsible. Similar irregularities in bacterial content were observed in the samples studied in the experiment next to be described. The increase in the mould count obtained in the sample stored at 75% R.H. showed that mould may grow on dehydrated fish and yet not to a sufficient extent to be visible to the naked eye. Except in the case of the sample stored at 70% R.H. there was a marked decrease in the viable bacteria in all cases after 102 days' storage. There were marked changes in the volatile base content of the stored samples. At humidities at which growth of bacteria and moulds occurred these changes could be due to formation of volatile bases (especially by bacteria), or to their utilization by the microörganisms. Volatilization of these basic compounds might also account for certain of the observed losses. There was a pronounced increase in the total volatile base content of the sample stored at 65% R.H., at which value the numbers of viable organisms had decreased. Trimethylamine developed in all samples, but most markedly in those stored at the lower humidities corresponding to moisture contents of about 11% and 12%.

Dehydrated lingcod was stored over sulphuric acid at between 10 and 80% R.H., analyses being made at given intervals. The results are recorded in table

TABLE III. Effect of storing over sulphuric acid solutions at different relative humidities on mould development and moisture, bacterial and volatile base content of dehydrated lingcod.

	Time of storage (days)	Initial values	Relative humidity (%)							
			10	20	30	40	50	60	70	80
Water content (%)	30	1.7	3.0	4.5	5.7	6.8	8.3	9.3	13.6	17.9
	97	1.7	3.2	4.3	5.5	7.2	8.5	9.2	13.4	16.8
Viable bacteria (colonies per g.)	30	4,700	7,400	4,900	6,300	9,800	66,000	4,100	4,600	900
	97	4,700	12,000	2,700	2,600	3,200	2,600	1,100	320	*
Total volatile base (mg. N per 100 g.)	30	27	24	24	29	28	28	28	12	11
	97	27	22	23	30	33	34	34	21	20
Trimethylamine (mg. N per 100 g)	30	4.2	2.2	4 2	8.4	8.4	12.6	4.8	4.8	3 1
	97	4 2	8 9	8 9	13	25	24	22	16	14

*Visible mould appeared after 76 days' storage. No bacterial colonies developed in the roll tubes, probably because their development was suppressed by the moulds which were very numerous
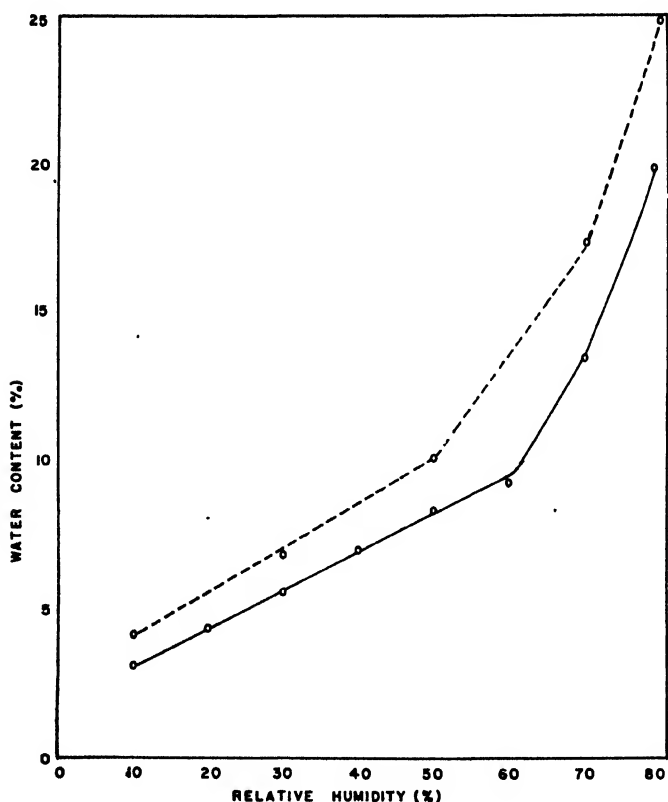
FIGURE 1. Relation between relative humidity and water content of precooked, air-dehydrated lingcod stored at 25°C. (continuous line) and of precooked, air-dehydrated beef stored at 37°C. (broken line; data taken from paper by Gane, 1943; water content calculated on fat-free basis).

III, the relation between R.H. and water content being shown in figure 1. It was found that the water content increased linearly with increasing humidity at 10 to 60% R.H., but that at higher humidities this relation was no longer maintained, the water content increasing sharply. In this respect the results parallel closely those obtained by Gane (1943) for pre-cooked, air-dehydrated beef (figure 1). There was slow development of visible mould at 80% R.H. (average moisture content 17.4%), and in this respect the results were identical with those obtained in the two previous experiments. If it is assumed that the high bacterial count obtained in the sample stored for 30 days at 50% R.H. was due to sampling error, it would appear that bacteria in dehydrated fish stored at 10 to 50% R.H. decrease only slightly, but that at higher humidities the decrease is more marked. The total volatile base had not increased in samples held for 30 days at 10 to 60% R.H.; there was, however, a definite decrease at 70 and 80% R.H. After 97 days only a slight increase in total volatile base was found at 30 to 60% R.H., while there was a slight decrease at 10, 20, 70 and 80% R.H. In this respect the results differed from those obtained in the foregoing experiment, and it is suggested that volatilization of ammonia and its neutralization by the sulphuric acid, over which the samples were stored, may have accounted for the failure of total volatile base to accumulate. The trimethylamine content of most samples increased after storage for 30 days, while after 97 days there was a marked increase in all cases, the accumulation being greatest at 40 and 50% R.H. Apparently under the conditions of storage trimethylamine did not volatilize as readily as ammonia.

LINGCOD, CHUM SALMON AND COHO SALMON STORED IN SEALED CANS

The water, volatile base and bacterial content of several samples of dehydrated fish were determined. Portions were then filled into ¼-lb. cans, sealed, and stored at 25°C. The results (table IV) showed that there was a pronounced increase in total volatile base and trimethylamine in samples with a water content of between 11.6 and 15.5%. On the other hand total volatile base did not increase, and trimethylamine increased only very slightly in samples with a water content of 1.7 to 5.4%. The data obtained from bacterial counts in the few samples examined were inconsistent.

TABLE IV. Moisture, bacterial and volatile base content of dehydrated fish stored in sealed cans. A is value obtained on the freshly dehydrated product; B is value obtained after the storage period given

Variety of fish	Time stored (days)	Water content		Viable bacteria (colonies per g.)		Total volatile base (mg. N per 100 g.)		Trimethylamine (mg. N per 100 g.)	
		A	B	A	B	A	B	A	B
Lingcod.......	97	1.7	1.8	4,700	11,000	27	27	4.2	8.9
Lingcod........	118	15.4	15.5	930	*	24	84	2.6	40
Coho salmon....	118	11.6	12.0	4,700	410	25	55	3.2	13
Chum salmon...	105	4.1	5.4	23,000	1,300	38	35	0	2.6
Chum salmon.	118	12.3	12.2	1,800	2,000	37	69	4.2	19

*36,000 moulds per gram; these presumably prevented growth of bacteria.

SUMMARY

Precooked fish, dehydrated in heated, moving air, became contaminated with bacteria, moulds and yeasts during preparation.

In samples of dehydrated fish stored at 25°C. visible mould developed only at 80% or higher R.H., corresponding to a water content of 16.8 to 18.3%. Mould increased to an extent not visible to the naked eye in samples stored at 75% R.H., corresponding to a water content of 13.2 to 13.6%. The R.H.—water content relation of dehydrated fish was similar to that of precooked, air-dehydrated beef.

In two samples of dehydrated fish stored at 85% R.H., corresponding to a water content of 19 to 19.7%, the numbers of viable bacteria decreased sharply, while in a third sample there was a slight apparent increase. At higher humidities bacteria multiplied rapidly, while at lower humidities the viable bacterial population decreased in general more slowly in the drier than in the moister samples.

Both total volatile base and trimethylamine increased markedly in dehydrated fish stored at certain humidities at which viable bacteria and moulds decreased.

REFERENCES

BATE-SMITH, E. C., C. H. LEA AND J. G. SHARP. *J. Soc. Chem. Ind.*, **62**, 100-104, 1943.

CUTTING, C. L. *Chem. and Ind.*, **61**, 365-368, 1942.

CUTTING, C. L., AND G. A. REAY. *Chem. and Ind.*, **63**, 47-50, 1944.

FALK, K. G., E. M. FRANKEL AND R. H. McKEE. *J. Ind. Eng. Chem.*, **11**, 1036-1040, 1919.

GANE, R. *J. Soc. Chem. Ind.*, **62**, 139-140, 1943.

HENRICI, A. T. Moulds, yeasts and actinomycetes, 1-296, Wiley, New York, 1930.

MACARA, T. J. R. *J. Soc. Chem. Ind.*, **62**, 104-106, 1943.

MARTINEK, W. A., AND B. JACOBS. *Fishery Market News*, **5**, 12-14, 1943.

STEVENSON, C. H. *Bull. U.S. Fish. Comm.*, **1898** (18) 337-563, 1899.

TARR, H. L. A. *Fish. Res. Bd. Can. Prog. Rep. Pac.*, **57**, 16-20, 1943.

TARR, H. L. A., AND B. E. BAILEY. *J. Fish. Res. Bd. Can.*, **4**, 327-336, 1939.

TRESSLER, D. K. Marine products of commerce. 1-762, Chem. Catalog. Co., New York, 1923.

YOUNG, L., AND C. F. LEE. *Fishing Gaz.*, **60**, 64-65 and 74, 1943.

YOUNG, O. C., AND E. P. SIDAWAY. *Fish. Res. Bd. Can. Prog. Rep. Pac.*, **56**, 7-9, 1943.

CANADIAN WILTSHIRE BACON

XXIV. EFFECT OF STRONG CURES ON KEEPING QUALITY[1]

By W. Harold White[2], N. E. Gibbons[3], and M. W. Thistle[4]

Abstract

The effect on keeping quality of hard curing by both wet and dry salting methods, and of treatment with borax, boric acid, acetylsalicylic acid, and a mixture of benzoic and citric acids was investigated on sides and gammons stored at 15.6° C. (60° F.) for periods up to 40 days. Of the methods studied for preventing spoilage, packing in a 13 : 3 salt–borax mixture was the most effective, but would be detrimental to flavour quality because of the presence of excessive quantities of sodium chloride and undesirable desiccation of the meat.

Experiments on the effect of surface bacterial contamination on internal spoilage indicated that deterioration in the deep meat was relatively independent of the condition of the surface.

Introduction

During the early war period an anticipated shortage of refrigerated space for the shipment of Wiltshire bacon to England prompted investigations on curing, smoking, and chemical preservative treatments that might prevent deterioration over extended periods at ordinary temperatures. The results of certain studies on smoking and chemical preservatives have been reported or are in press (9, 11, 12, 13). The present paper describes four experiments on the suitability of strong cures and other supplementary treatments for maintaining quality at elevated temperatures. In addition, observations on the effect of surface bacterial contamination on internal spoilage of bacon are given.

I. Tank Curing and Surface Treatment with Borax and Boric Acid

Procedure

It was desired to ascertain the relative effect of strong, or so-called 'hard', tank cures, borax, and boric acid, on keeping quality. Since a saturated solution of sodium chloride is normally employed in the curing of Canadian Wiltshire bacon, it was necessary to use other methods to obtain the required

---

[2] *Formerly Biochemist, Food Investigations; at present, Chemist, Imperial Oil Co. Ltd., Sarnia, Ont.*

[3] *Bacteriologist, Food Investigations.*

[4] *Biochemist, Food Investigations.*

---

hard-cured product. Of the available methods extra pumping, overhauling, and increasing the ratio of pickle to meat were considered most suitable for use in this study. Three sides, taken from different hogs, were cured by each of the following procedures: (i) hard—commercial pumping pickle and procedure; cured four days in a small tank; overhauled; repumped; and cured for a further four days; (ii) medium—commercial pumping pickle and procedure, and cured eight days in a small tank; and (iii) regular—as for (ii) but cured eight days in a large commercial tank. The meat to pickle ratio in the small tank was approximately 5 lb. of meat to 1 gal. of pickle as compared to the usual proportion of about 20 lb. to 1 gal.

After removal from cure the sides were drained for one day at 3.3° C. (38° F.) and wiped, prior to baling. The meat surface of three sides, representing each of the types of cures, was dusted with 1 lb. of borax, another set of three each with 1 lb. of boric acid, and the remainder left untreated. Thus all combinations of cure and surface treatment were studied. The three sides given the same surface treatment were baled together so that meat to meat surfaces were together for two of the sides, and meat to skin for the third. The bales were stored at 15.6° C. (60° F.) for 32 days, conditions considered to be comparable to those encountered in shipment as stowage. The entire experiment was duplicated with a second group of nine sides.

In tests of this nature it was considered unnecessary to adjust the quantities of borax and boric acid to a comparable basis in terms of boron, since treatments of 1 lb. per side represented an excess of both compounds. Moreover, losses from the surface due to mechanical action, subsequent drainage of the solution formed with the meat juices, and the differential solubility of the two compounds vitiate comparisons based on boron content.

Bacteriological, chemical, and visual examinations of the sides were made at the end of the storage period. Chloride, nitrate, nitrite (7) and, in certain instances, borax or boric acid determinations (1, p. 460) were made on a slice of gammon approximately 1.25 in. thick, removed from immediately in front of the round bone, and on a sample of the back between the third and sixth ribs.

Differences in the sodium chloride content and, for certain samples, the borax and boric acid content, between the inside position adjacent to the fat and the surface layer were determined on a central strip of a slice of the gammon, approximately 1.5 in. in thickness, containing the round bone (10). Chloride was determined by a wet oxidation method (6). Peroxide oxygen determinations were made on the gammon fat (8).

Bacteriological counts were made of the deep meat of all gammons, and the rib surfaces for most of the sides. The deep-meat samples were taken from a slice of the gammon approximately 2 in. thick that had been flamed on both sides until charred. Several cores approximately 0.5 in. in diameter were obtained with a stainless steel cork borer, transferred to a Petri dish, and the charred and coagulated meat from each core removed. A sample of about 5 gm. was ground with sand, diluted with a 4% sodium chloride solution, and plated on 4% salt agar. Counts were made after incubation for six days at

20° C. Surface counts were made on samples of 9 to 12 sq. cm. of rib surface by a procedure described previously (3).

RESULTS

Visual Examination

Visual examination of the sides after storage for 11 days at 15.6° C. showed them to be in a reasonably satisfactory condition. After 32 days' storage no obvious differences could be detected between the three cures (Table I). Of

TABLE I

EFFECT OF TYPE OF CURE AND SURFACE TREATMENT ON THE VISIBLE APPEARANCE AND CONDITION OF WILTSHIRE SIDES AFTER STORAGE FOR 32 DAYS AT 15.6° C.

Type of cure[1]	Surface treatment		
	None	Borax	Boric acid
Hard	Very slimy; gammon and pocket sour; very moist. Poor condition.	Slime; excess salt; moist; pocket sour. Fair condition.	Slight slime on ribs; mould; slight excess salt; moist; fore end slightly sour. Fair condition.
Medium	Very slimy; gammon and pocket sour; very moist; one side with gas on ribs and blue stain. Poor condition.	No visible slime; excess salt; slightly moist; one gammon gassy and sour. Fair condition.	Slight slime on ribs; mould; slight excess of salt; slightly moist. Fair condition.
Regular	Very slimy; gammon sour; very moist; one side with blue stain on ribs; surface sour. Poor condition.	Slime; excess salt; moist; gammon sour. Fair condition.	Slight slime on ribs; moulds; slight excess of salt; slightly moist. Fair condition.

[1] *As defined in the text.*

the surface treatments boric acid appeared to be more effective than borax. The colour of all the sides was good. All the bales were very wet. The regions of the blade pocket and around the bones appeared to be most vulnerable to spoilage. While the odour of the freshly cut surface of none of the sides was putrid, it was not prime, and suggested the occurrence of incipient changes.

Chloride Content and Distribution

The average chloride content of the hard-cured backs and gammons was greater than that of the medium- or regular-cured product, but the differences were smaller than expected (Table II). A satisfactory distribution of chloride in the sides was obtained for all three sides. Additional pumping effectively introduced sodium chloride into the deeper portions of the gammon.

Nitrate Content

The hard-cured sides contained the most nitrate (Table III). There was little difference between the amount in the back and the gammon. Since a

similar conclusion was noted for the chloride content, it would appear that the pumping and curing procedures employed gave satisfactory distribution of salts in the sides.

TABLE II

EFFECT OF METHOD OF CURE ON THE CONTENT AND DISTRIBUTION OF SODIUM CHLORIDE IN WILTSHIRE BACKS AND GAMMONS AFTER STORAGE FOR 32 DAYS AT 15.6° C.

Type of cure[1]	Sodium chloride, %			
	Back	Gammon	Distribution in gammon[1]	
			Inside	Outside
Hard	5.6	6.1	9.0	8.7
Medium	5.5	5.6	7.7	8.1
Regular	5.4	5.3	7.4	7.6

[1] *As defined in the text.*

TABLE III

EFFECT OF METHOD OF CURE ON THE CONTENT OF SODIUM NITRATE IN WILTSHIRE BACKS AND GAMMONS AFTER STORAGE FOR 32 DAYS AT 15.6° C.

Type of cure[1]	Sodium nitrate, %	
	Back	Gammon
Hard	0.27	0.31
Medium	0.19	0.24
Regular	0.22	0.23

[1] *As defined in the text.*

Nitrite Content

While the mean nitrite content of the gammons over all surface treatments increased with increase in the hardness of the cure, that of the backs decreased (Table IV). The mean nitrite content of both backs and gammons over all cures was greatest for sides treated with borax, less for those given no surface treatment, and least for those on which boric acid had been dusted. This must be attributed primarily to differential growth of nitrate-reducing micro-organisms, since the magnitude of the differences is such that they cannot be accounted for directly by variable pumping procedures. It would appear that the growth of nitrate-reducing micro-organisms was little affected by borax but was retarded by boric acid.

In most instances the mean nitrite content was high. This suggests that the concentrations of nitrate employed in Canadian standard Wiltshire curing pickles should be reduced if bacon is to be held at 15.6° C. for one month without approaching or exceeding the legal limit of nitrite (200 p.p.m.) (2).

TABLE IV

EFFECT OF TYPE OF CURE AND SURFACE TREATMENT ON THE CONTENT OF SODIUM NITRITE IN WILTSHIRE BACKS AND GAMMONS AFTER STORAGE FOR 32 DAYS AT 15.6° C.

Type of cure[1]	Surface treatment			
	None	Borax	Boric acid	Mean
	Sodium nitrite, p.p.m.			
Gammon				
Hard	296	664	90	350
Medium	257	281	157	232
Regular	208	404	15	209
Mean	254	450	87	264
Back				
Hard	59	216	83	119
Medium	173	251	89	171
Regular	139	453	7	200
Mean	124	307	60	163

[1] *As defined in the text.*

Content and Distribution of Borax and Boric Acid

Both the backs and gammons, on the average, contained more boric acid than borax (Table V), a reflection of the relative solubilities of the two compounds. The concentration of borax and boric acid in the gammons was approximately four times greater at the external than at the inside sampling positions. Although the quantities of borax and boric acid observed here are not toxic, it should be recalled that boron acts as a cumulative poison in the human body.

TABLE V

CONTENT AND DISTRIBUTION OF BORAX AND BORIC ACID IN WILTSHIRE BACKS AND GAMMONS AFTER STORAGE FOR 32 DAYS AT 15.6° C.

Borax, %				Boric acid, %			
Back	Gammon	Distribution in gammon		Back	Gammon	Distribution in gammon	
		Inside	Outside			Inside	Outside
0.45	0.35	0.24	0.97	0.48	0.72	0.35	1.33

Peroxide Oxygen Content

The mean peroxide oxygen content of the gammon fat, averaged over all surface treatments, progressively decreased from the hard-cured to the regular-cured product (Table VI). It would appear that the addition of

TABLE VI

EFFECT OF TYPE OF CURE AND SURFACE TREATMENT ON THE PEROXIDE OXYGEN CONTENT OF
WILTSHIRE GAMMON FAT AFTER STORAGE FOR 32 DAYS AT 15.6° C.

Type of cure[1]	Surface treatment			
	None	Borax	Boric acid	Mean
	Peroxide oxygen content, ml. 0.002 N Na$_2$S$_2$O$_3$			
Hard	7.4	2.6	1.5	3.8
Medium	2.5	2.9	1.4	2.3
Regular	2.0	1.3	1.4	1.6
Mean	4.0	2.3	1.4	2.6

[1] *As defined in the text.*

further quantities of curing salts was associated with a decrease in the stability of the fat. The mean peroxide oxygen content, as averaged over all types of cures, was greatest for the control, less for borax and least for boric-acid–treated sides. This may be a reflection of the relative effects of the various treatments on the growth of micro-organisms elaborating oxidizing enzymes.

Bacterial Counts

The surface bacteriological counts were high and indicate either the presence or imminent appearance of slime (Table VII). While slime could be readily observed on the untreated surfaces, it was difficult to detect on those dusted with borax or boric acid. The type of cure had little effect on surface bacterial growth. The mean count for the sides treated with borax was slightly higher than that of the controls, showing that borax had little or no bactericidal or bacteriostatic action on surface organisms. Other experiments have indicated as great or greater bacterial development on bacon treated with borax as on controls (4). It is believed that this is due to a raising of the pH of the meat to a level more favourable to bacterial development. The use of boric acid effected some decrease in the mean surface count.

In the deep-meat counts (Table VII) a distinction was made between the large, easily seen type of colony, such as appeared on surface plates, and the small, pin-point types that are just discernible with the naked eye and must be counted at a magnification of 10 or 15×. The mean number of large and of pin-point colonies was approximately the same for all types of cure and surface treatments, and showed little relation to the surface count. Pin-point counts were on the average 1000 times higher than those for the large colonies.

TABLE VII

EFFECT OF TYPE OF CURE AND SURFACE TREATMENT ON SURFACE AND DEEP-MEAT BACTERIO-
LOGICAL GROWTH IN WILTSHIRE BACON AFTER STORAGE FOR 32 DAYS AT 15.6° C.

Type of cure	Surface treatment			
	None	Borax	Boric acid	Mean
Surface				
	Log$_{10}$ number of organisms per sq. cm.			
Hard	8.99	9.05	—	—
Medium	8.17	9.04	6.04	7.75
Regular	8.38	8.80	6.16	7.78
Mean	8.51	8.96	6.10	—
Deep-meat—(a) Large colonies				
	Log$_{10}$ number of organisms per gm.			
Hard	3.43	3.79	3.89	3.70
Medium	3.78	3.00	4.03	3.60
Regular	3.32	3.02	3.39	3.24
Mean	3.51	3.27	3.77	3.51
Deep-meat—(b) Pin-point colonies				
	Log$_{10}$ number of organisms per gm.			
Hard	7.21	6.63	6.74	6.86
Medium	6.12	6.48	7.00	6.53
Regular	7.16	6.95	6.21	6.77
Mean	6.83	6.69	6.65	6.72

II. Combined-Tank and Dry Curing; Drainage Time; and Surface Treatment with Benzoic and Acetylsalicylic Acids

PROCEDURE

The experiment was designed to obtain information on the effect of combined tank and dry-salt curing, drainage time, and surface treatment with benzoic acid and acetylsalicylic acids (11) on the keeping quality of bacon. For this purpose the right and left sides of four hogs were pumped and cured, using standard Wiltshire curing pickles, and drained, as shown in Table VIII. The shoulders were removed, and the meat surface of the remaining portion of the sides dusted either with a mixture of 31.5 gm. of benzoic acid and 15.5 gm. of citric acid or with 31 gm. of acetylsalicylic acid. After baling, the sides were stored at 15.6° C. (60° F.), and visually examined after 16 and 32 days.

TABLE VIII

EFFECT OF COMBINED TANK AND DRY CURING, DRAINAGE TIME, AND SURFACE TREATMENT WITH BENZOIC AND ACETYLSALICYLIC ACIDS ON CONTENT AND DISTRIBUTION OF CURING SALTS IN, AND BACTERIAL GROWTH ON, WILTSHIRE BACON AFTER STORAGE FOR 32 DAYS AT 15.6° C.

Side No.	Curing treatment	Drainage time, days	Surface treatment[1]	Back NaCl, %	Back NaNO2, p.p.m.	Gammon NaCl, %	Gammon NaNO2, %	Gammon NaNO2, p.p.m.	Salt distribution in gammon NaCl, % I	NaCl, % O	NaNO2, % I	NaNO2, % O	NaNO2, p.p.m. I	NaNO2, p.p.m. O	Surface bacterial count, log10 no. organisms per sq. cm. Large colonies	Pin-point colonies
1 R	Tank, 8 days	0	Benzoic acid	—	—	—	—	—	—	—	—	—	—	—	3.90	0
2 R	Tank, 8 days	0	Acetylsalicylic acid	—	—	—	—	—	—	—	—	—	—	—	6.63	0
3 L	Tank, 8 days	1	Benzoic acid	5.72	0.07	18	6.08	0.07	18	6.04	5.73	0.07	0.06	12	33	6.60	0
4 L	Tank, 8 days	1	Acetylsalicylic acid	6.20	0.12	28	5.52	0.09	32	5.82	5.66	0.11	0.13	14	33	6.87	0
1 L	Tank, 8 days	3	Benzoic acid	—	—	—	—	—	—	—	—	—	—	—	5.31	6.38
2 L	Tank, 8 days	3	Acetylsalicylic acid	—	—	—	—	—	—	—	—	—	—	—	6.48	0
3 R	Tank, 5 days; dry salt, 4 days	0	Benzoic acid	7.01	0.05	36	6.32	0.09	24	7.12	7.42	0.09	0.09	17	31	5.92	6.72
4 R	Tank, 5 days; dry salt, 4 days	0	Acetylsalicylic acid	6.20	0.07	7	7.00	0.15	39	7.22	7.06	0.17	0.13	22	6	9.16	0

I: *Position adjacent to fat.*

O: *Position adjacent to surface.*

[1] *See text.*

At the termination of the storage period, surface bacterial counts were made on all sides, and the content of curing salts in the backs and gammons determined for four of the sides. Measurements were also made of the distribution of curing salts in the gammons. The methods employed have been described in Part I.

RESULTS

No definite changes could be observed after 16 days' storage. However, at 32 days, 3L had a generally sour odour and 1L, 2R, 2L, and 3R were slightly sour in areas adjacent to the round bone (Table VIII). Slime was very heavy on Side 4R, while on the others it was mainly localized around the bones. Moreover, the skin was quite slimy on those sides that were at the bottom of the bales and had remained moist. There was little difference between acetylsalicylic acid and the mixture of benzoic and citric acids as preventatives of slime. However, while moulds were present on all sides, they were especially prevalent on those treated with acetylsalicylic acid.

The results of the chemical analyses on the meat are given in Table VIII. The salt content of the sides given the combined curing treatment was slightly greater than that of sides cured in the tank only, but the differences were smaller than expected and would not merit the extra processing costs involved.

III. Dry Salting of Wiltshire Gammons

PROCEDURE

The investigation was undertaken to assess the suitability of a suggestion often made that Wiltshire bacon could be shipped satisfactorily under stowage conditions if packed with dry salt and borax in wooden boxes. The material studied consisted of four Wiltshire gammons from different hogs, taken over the available size range. To simulate attachment to the original sides, the cut surfaces were dipped in melted vegetable shortening, and further protected by a double thickness of waxed paper. After an initial sampling, two of the gammons were placed in a wooden box and packed in a 13 : 3 salt–borax mixture. The top layer, separated by waxed paper, consisted of the remaining two gammons, which were thus available for interim sampling. One pound of the preservative mixture was used to four pounds of meat. The gammons were stored at 15.6° C. for 40 days, conditions considered to be comparable to a stowage environment.

Differences in the sodium chloride and moisture content between the inside position adjacent to the fat and the outer layer were determined during the storage period at 10-day intervals. For this purpose, two 0.75 in. borings were taken from sites chosen at random on the exposed meat surface, leaving space for the two final cross-sectional slices mentioned below. In order to avoid increased surface exposure of the gammons to the salt mixture, the holes were filled with solid vegetable shortening. Additional preservative was necessary to reproduce initial conditions after each sampling.

The distribution of salt, moisture, and borax at the beginning and end of storage was determined by the central strip method, described in Part I, on slices of the gammon removed from each of two positions, A, near the butt end and B, near the round bone. Additional slices from each of the two positions were combined in order to obtain some representation of the entire gammon. These complete samples were analysed for their chloride, moisture, and borax content. Spoilage in the gammon fat was assessed by determination of the peroxide oxygen content. Deep-meat bacterial counts were made on the central sections of the two undisturbed gammons.

The methods employed for determination of the deep-meat bacterial counts, and the salt, borax, and peroxide oxygen content have been described in Part I. The moisture content of the meat was determined by drying 2- to 3-gm. samples *in vacuo* for 16 hr. at 100° C.

RESULTS

Organoleptic inspection indicated the freshly-cut meat surfaces to be still in fair condition after 40 days' storage. The surfaces directly exposed to the salt mixture were dark in colour, hard and dry, with no evidence of moulds or slime.

The distribution of salt and moisture after 0, 20, and 30 days' storage is shown in Table IX. As expected, the salt content increased with storage

TABLE IX

AVERAGE CHANGES IN THE DISTRIBUTION OF SALT AND MOISTURE DURING STORAGE AT 15.6° C. OF TWO WILTSHIRE GAMMONS IN A SALT–BORAX MIXTURE

Storage time, days	Sodium chloride, %		Moisture, %	
	Inside	Outside	Inside	Outside
0	2 46	4 68	75 0	67 1
10	4 92	7 62	70 2	65 9
20	7 41	10 43	68 2	59 5
30	8 82	12 16	65 4	54 9

time, while the moisture content decreased. Improvement in distribution with storage was slight. Comparison with Tables X and XI shows that the small borings employed do not represent this type of material with any great efficiency. However, they are of value in indicating trends, as in the present instance.

The results for the distribution of chloride, moisture, and borax at the beginning and end of the storage period are presented in Table X. The salt gradient at the end of storage, while better than it was initially, is not regarded as particularly satisfactory. The salt level differed both across and along

TABLE X

Distribution of salt, moisture, and borax in Wiltshire gammons at the beginning and end of storage at 15.6° C for 40 days in a salt–borax mixture

Storage time, days	Sample No.	Sodium chloride, %				Moisture, %				Borax, %			
		Position A[a]		Position B[a]		Position A		Position B		Position A		Position B	
		Inside[a]	Outside[a]	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside
0	1	—	—	1.83	8.09	—	—	70.0	65.3	—	—	—	—
	2 and 3	—	—	3.34[1]	5.83	—	—	73.0	68.3	—	—	—	—
	4	—	—	1.45	4.73	—	—	78.4	69.3	—	—	—	—
40	1	9.65	12.39	11.90	13.90	61.6	56.6	60.0	55.4	0.17	0.46	0.25	0.72
	2 and 3	7.30[1]	9.52	8.33	10.20	68.6	62.1	68.4	63.9	0.18	0.38	0.19	0.42
	4	7.14	9.81	8.78	10.40	68.2	61.2	65.2	62.2	0.13	0.39	0.16	0.47

[1] *Values averaged for Sample Nos. 2 and 3.*
[a] *See text.*

TABLE XI

Chemical and bacteriological measurements on Wiltshire gammons after 40 days' dry salting at 15.6° C.

Sample No.	Sodium chloride, %	Moisture, %	Borax, %	Peroxide oxygen content of fat, ml. 0.002 N $Na_2S_2O_3$	Deep meat bacterial content, $\log_{10}$ no. per gm.	
					Large colonies	Pin-point colonies
1	12.71	57.6	0.43	0.94	3.24	6.38
2 and 3	9.02[1]	65.6	0.29	2.25	—	—
4	9.67	64.1	0.36	1.82	2.38	6.30

[1] *Values averaged for sample Nos. 2 and 3.*

the gammons, i.e. between the inside and outside layers and Positions *A* and *B*. Moisture distribution was as expected, the outer layer being about 5% drier than the inner. When borax was mixed with salt, borax distribution was less than half that when used alone (cf. Table V).

The over-all chloride, moisture, and borax contents of the gammons after storage are given in Table X. The salt content is excessive for satisfactory flavour quality. Depending on gammon size, dry-salting introduced from 50 to 100% more sodium chloride than the hardest cures described in Part I. Moreover, it was accompanied by excessive drying of the meat. The total borax content differed only slightly from that obtained when applied alone to the surface (cf. Table V).

The condition of the fat, as assessed by the peroxide oxygen content, was regarded as satisfactory (Table XI).

The numbers of large and pin-point bacterial colonies after storage (Table XI) were much the same as found after 32 days' storage of the hard, tank-cured product studied in Part I (cf. Table VII).

Packing bacon in a mixture of dry salt and borax would appear to have some preservative effect, but it is detrimental to flavour quality. The dry-salt pack not only introduces excessive quantities of sodium chloride but also causes undesirable drying.

IV. Dry Salting of Wiltshire Sides in Bales

PROCEDURE

In the previous study it was observed that a high salt concentration in the meat retarded spoilage, but would be detrimental to flavour quality. The object of the present experiment was to ascertain if a high surface and lower internal salt concentration would sufficiently protect bacon from spoilage, and at the same time give a more palatable product.

The right and left sides of two hogs were used for this purpose. Each side was pumped with 19 stitches, but only half the usual stroke, the minimum pumping treatment considered to be practical. One side from each hog was wiped, and the blade pocket and hollow of the ribs filled with salt. Two sides were placed with meat surfaces together so that all the intervening space was filled with salt. After rubbing the skin surface of the sides with salt, they were baled and stored at 15.6° C. for 32 days. The remaining two sides were treated in the same manner except that they were cured for two days in a commercial tank prior to dry salting.

The quality of the sides after storage was assessed by organoleptic examination; determination of the chloride, nitrate, nitrite, and moisture contents of the gammons and backs, and salt distribution in the gammons; and by surface bacterial counts on the backs and deep-meat counts on the gammons. The analytical methods and sampling procedures were the same as described previously except that the surface counts were determined by diluting with both 4 and 10% brine and plating on agars of similar salt concentrations.

RESULTS

The changes in weight of the sides during cure and storage are of some interest. The increase on pumping was uniform and about half the usual amount of 5%. The average, over-all loss in weight of the dry salted sides was 9.9%, and 8.6% for the sides that had been partially tank-cured.

All of the sides had slimy, putrid areas on the skin surface where there was little or no salt. The surface colour of the entirely dry cured sides was dull and brownish as a result of methaemoglobin formation, while that of the partially tank-cured sides was satisfactory. Mould and slime were evident on some sides where the salt had fallen off. The odour of the freshly-cut surface of the gammons of the partially tank-cured sides was somewhat sour and of those entirely dry-salted, definitely off.

The deep-meat bacterial counts were variable, but all showed a considerable number of pin-point colonies (Table XII). There were usually more surface bacteria on the tank-cured than on the dry-salted sides. However, in all

TABLE XII

Number of bacteria on the surface and in the deep meat of Wiltshire
sides, dry salted in bales for 32 days at 15.6° C.

Cure	Side No.	Surface organisms, $\log_{10}$ no. per sq. cm. as determined on agar containing:		Deep-meat organisms, $\log_{10}$ no. per gm. on 4% salt agar	
		4% Salt	10% Salt	Large colonies	Pin-point colonies
Tank + dry salt	1 R	6.33	5.63	3.51	7.30
	2 L	4.65	5.16	2.66	7.42
Dry salt	1 L	3.72	3.86	3.96	6.15
	2 R	4.73	4.62	2.70	7.58

instances the number of these organisms was considerably below the level
at which slime is usually observed.

The results of the chemical analyses are given in Table XIII. The content
of sodium chloride was relatively high in all sides. The tank-cured sides
contained somewhat more nitrite, but the differences were small and probably
would not account for the variations in keeping quality observed between the
two types of cures. The difference in chloride content between the interior
and surface of the gammons, amounting to about only 2%, was smaller than

TABLE XIII

Content and distribution of curing salts and moisture in Wiltshire sides,
dry salted in bales for 32 days at 15.6° C.

Cure	Side No.	Position of sample[1]	Sodium chloride, %	Sodium nitrate, %	Sodium nitrite, p.p.m.	Moisture, %
Tank + dry salt	1 R	Inside	9.70	0.14	46	67.6
		Outside	11.83	0.06	14	58.0
		Gammon	10.65	0.08	10	60.6
		Back	4.90	0.01	3	62.1
	2 L	Inside	5.58	0.05	6	72.1
		Outside	7.48	0.03	63	66.8
		Gammon	6.22	0.03	6	67.1
		Back	6.57	0.04	7	61.2
Dry salt	1 L	Inside	8.18	0.08	5	71.0
		Outside	9.41	0.04	10	62.6
		Gammon	8.91	0.02	3	64.2
		Back	6.32	0.02	2	61.6
	2 R	Inside	6.34	0.05	5	69.9
		Outside	8.34	0.07	6	64.8
		Gammon	7.06	0.02	3	65.4
		Back	7.06	0.01	1	58.4

[1] *See text.*

expected. However, in spite of this, it would appear that the maintenance of a high salt concentration at the surface alone is not sufficient to prevent internal spoilage of Wiltshire bacon.

V. Effect of Surface Bacterial Contamination on Internal Spoilage

Three experiments were made to ascertain if internal spoilage in bacon was related to surface bacterial growth and the subsequent diffusion of elaborated enzymes into the meat.

Experiment 1

In the first experiment the relative effectiveness of salt contents of approximately 4 and 10% in retarding spoilage in and on the surface of bacon was studied. For this purpose the longissimus dorsi muscle was removed from a Wiltshire-cured back, and cut into four pieces of equal length. The two central portions were injected with 10 ml. of a saturated brine, and immersed in a brine of similar concentration for 72 hr. at 4.4° C. (40° F.). To simulate the interior of a side, one piece of high and another of low salt content were placed in the bottom of a vessel and covered with melted vegetable shortening. After the fat had hardened the remaining two pieces were placed on top and fat added until they were half covered. The vessel was covered and stored for 28 days at 15.6° C. In this manner bacon of both high and low salt content was exposed to, and excluded from, air.

Organoleptic examination of the surface samples after 14 days' storage showed that of high salt content to be in good condition, and the other to be fairly slimy with some mould growth, but no off-odours. At the end of the storage period, the exposed surfaces of the samples, which had been partially immersed in the fat, had off-odours and were covered with bacteria and some moulds. In contrast, the samples covered with fat during storage had no visible growth on the surface, but the piece containing the lesser amount of chloride had a sour odour.

The chloride and nitrite content of each sample was determined by methods described previously. The average difference between samples of low and high salt content was somewhat larger than expected (Table XIV). The nitrite content was highest in the samples that had been partially exposed to the atmosphere.

Samples for deep-meat bacterial counts were taken from the centre of each piece. The plates were incubated under both aerobic and anaerobic conditions. The most striking observation was the absence of pin-point colonies in meat containing 12 to 13% salt (Table XIV). Moreover, this type of colony was more prevalent in the partially exposed pieces of meat than in those buried in fat.

It would appear that a salt concentration as high as 13% is not sufficient to prevent surface spoilage but has some effect on deterioration in the deep meat. Internal spoilage is not due to enzymes diffusing into the meat from the

TABLE XIV

EFFECT OF SALT CONTENT AND EXPOSURE TO AIR ON THE NITRITE CONTENT, AND DEEP-MEAT,
AEROBIC, AND ANAEROBIC BACTERIAL COUNTS OF BACON
STORED AT 15.6° C. FOR FOUR WEEKS

Sample No.	Treat-ment	Content of curing salts		Bacterial count, $\log_{10}$ no. per gm.			
				Aerobic		Anaerobic	
		NaCl, %	NaNO₂, p.p.m.	Large colonies	Pin-point colonies	Large colonies	Pin-point colonies
1	Covered	3.13	5	2.04	5.88	1.95	5.78
4	Covered	12.84	71	2.40	1.23	2.23	0
3	Exposed	5.72	220	1.97	7.46	1.70	7.45
2	Exposed	13.26	134	3.68	0	2.92	0

surface since the buried piece containing 3% salt had no visible surface growth and yet was spoiled internally. This conclusion is based on the assumption that appreciable diffusion of enzymes into the meat would occur only if the surface bacterial content was very high.

Experiment 2

The interior of a ham is essentially anaerobic. Since many anaerobes are inhibited by 4% salt, it has been suggested that spoilage of bacon is due to the growth of organisms on the surface, with the subsequent diffusion of enzymes into the deeper tissues. If this hypothesis is correct, it might reasonably be assumed that deterioration would be retarded if the meat were stored under anaerobic conditions, provided that the surface flora were aerobic or of the same type as present in the deep meat.

A Wiltshire gammon was stored in a desiccator under anaerobic conditions, obtained with hydrogen and palladinized asbestos, for four weeks at 21° C. (70° F.).

After storage the surface condition of the meat was poorer than when treated similarly under aerobic conditions. The ham had a bright red colour and a putrid odour. There was slime over most of the surface and some blue discoloration of the fat. The interior of the ham was likewise spoiled.

Samples (15 sq. cm.), taken from the surface for bacterial counts were plated on both nutrient and 4% salt agar, and incubated aerobically at 19.9° C. (68° F.) and anaerobically at room temperature. Deep-meat aerobic and anaerobic counts were made on a slice from the centre of the ham. These were plated on 4% salt agar only.

The results are given in Table XV. The presence of pin-point colonies on the surface is of interest. These were not usually found by aerobic methods, but since they were evident also after anaerobic incubation they are undoubtedly present on the surface. Storage under anaerobic conditions apparently accelerated rather than retarded surface spoilage of bacon. Hence

TABLE XV

NUMBER OF BACTERIA ON THE SURFACE AND IN THE DEEP–MEAT OF A WILTSHIRE
GAMMON STORED ANAEROBICALLY FOR FOUR WEEKS AT 21° C.

Method of incubation	Surface counts, $\log_{10}$ no. per sq. cm.				Deep-meat counts, $\log_{10}$ no. per gm.	
	Nutrient agar		4% Salt agar		4% Salt agar	
	Large colonies	Pin-point colonies	Large colonies	Pin-point colonies	Large colonies	Pin-point colonies
Aerobic	8.31	0	8.34	Few	6.39	0
Anaerobic	8.28	Few	8.52	7.91	6.47	7.67

surface spoilage is not directly responsible for deterioration in the meat. The surface flora must consist essentially of facultative anaerobes.

Experiment 3

The study was designed to determine the effect on keeping quality of varying the internal bacterial content of bacon, while restricting surface growth to the lowest level possible. For this purpose 18 hams from different hogs were vein pumped with six curing pickles differing with respect to both the number and type of bacteria. The pickles were prepared by dividing a quantity of commercial curing solution into six equal portions, and subsequently sterilizing five of these. Organisms from sides and from pumping pickles were grown on nutrient agar and on agars containing 4 and 10% salt. Emulsions of these organisms were made in 10% brine, and used for inoculating four of the sterile solutions to give pump pickles of low and high contents of slime and pickle organisms. Bacterial counts for the pickles are given in Table XVI.

After pumping, the hams were cured in a commercial tank for seven days. To reduce the number of bacteria on the surface they were painted with a

TABLE XVI

BACTERIAL COUNTS OF PUMP PICKLES INOCULATED WITH VARIOUS AMOUNTS
OF SLIME AND PICKLE ORGANISMS

Pickle	$\log_{10}$ no. of bacteria per ml. as determined on:		
	Nutrient agar	4% Salt agar	10% Salt agar
Sterile	0.00	0.00	0.00
Low slime[1]	—	—	—
High slime	6.37	7.06	6.81
Low pickle	2.93	3.31	3.29
High pickle	6.38	6.97	6.92
Regular	2.81	3.29	3.19

[1] *Plates misplaced.*

4% aqueous solution of formaldehyde. They were then dried for three hours at room temperature, wrapped, and stored at 15.6° C. for 28 days. The formaldehyde treatment was repeated at weekly intervals during storage.

At the end of storage, surface bacterial counts were made on one ham selected at random from the three available for each pumping treatment. Two slices of each ham were taken for determination of deep-meat bacterial counts, and the chloride and nitrite content. In addition the formaldehyde content of a slice of the meat at positions adjacent to the fat and surface was determined for four hams (5).

There was no slime on any of the hams after storage. The odour of the internal meat of all hams was slightly sour.

Data for the surface and deep-meat bacterial counts are given in Table XVII. The surface counts were low. The number of large colonies in the deep meat more or less paralleled the bacterial content of the pump pickles employed. Based on a 4% increase in weight on pumping, the number was usually lower than would be expected, and indicates that appreciable growth

TABLE XVII

BACTERIAL AND CHEMICAL ANALYSES OF WILTSHIRE GAMMONS INOCULATED WITH VARIOUS
AMOUNTS AND TYPES OF BACTERIA AND STORED AT 15.6° C. FOR FOUR WEEKS

Pickle	Sample No.	Surface, $\log_{10}$ no. per sq. cm.	Deep-meat, $\log_{10}$ no. per gm.		Sodium chloride, %	Sodium nitrite, p.p.m.	Formaldehyde, %	
			Large colonies	Pin-point colonies			Inside[1]	Outside
Sterile	1	2.16	2.76	5.71	6.26	52	0.07	0.09
	2	—	0.95	5.65	4.53	76	—	—
	4	—	1.00	6.70	5.73	41	—	—
Low slime	5	—	2.70	6.64	7.82	43	0.08	0.10
	6	—	2.23	4.97	5.98	48	—	—
	7	2.18	3.03	6.58	6.43	29	—	—
High slime	9	3.30	5.33	6.35	5.52	24	—	—
	11	—	5.45	7.51	6.07	136	—	—
	17	—	4.31	6.39	6.63	126	—	—
Low pickle	3	—	2.29	5.26	7.70	134	—	—
	8	—	1.26	7.26	6.08	45	—	—
	16	2.58	1.48	6.51	5.70	46	—	—
High pickle	12	—	3.40	7.34	7.02	276	0 12	0.17
	13	1.83	5.78	7.48	6.73	86	—	—
	10	—	4.18	7.56	5.48	91	—	—
Regular	14	—	1.64	6.18	6.78	65	—	—
	15	1.60	1.48	6.41	5.78	76	—	—
	18	—	1.15	6.40	6.38	41	0.13	0.15

[1] *See text.*

did not occur under the conditions of the experiment. The number of deep-meat, pin-point colonies was in all instances high, and especially in hams pumped with the brine that had been heavily inoculated with pickle organisms.

The results of the chemical analyses are shown in Table XVII. There was some indication that the nitrite content varied directly with that of bacteria. The content of about 0.1% formaldehyde in the meat adjacent to the fat is surprisingly high since it was considered that little diffusion would occur under the experimental conditions employed.

Although bacterial growth on the surface was prevented, spoilage occurred in the interior of the hams. However, the extent of deterioration was less than normally occurs. This was presumably due in part to the presence of appreciable quantities of formaldehyde in the meat.

Acknowledgments

References

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis. 5th ed. A.O.A.C., Washington, D.C. 1940.
2. CANADA. DEPT. OF PENSIONS AND NATIONAL HEALTH. Regulations under the foods and drugs act. 1942.
3. GIBBONS, N. E. Can. J. Research, D, 18 : 202-210. 1940.
4. GIBBONS, N. E. Unpublished data.
5. HIGHBERGER, J. H. and RETSCH, C. E. J. Am. Leather Chem. Assoc. 33 : 341-352. 1938.
6. KERR, R. H. J. Assoc. Official Agr. Chem. 16 : 543-546. 1933.
7. WHITE, W. H. Can. J. Research, D, 17 : 125-136. 1939.
8. WHITE, W. H. Can. J. Research, D, 19 : 278-293. 1941.
9. WHITE, W. H. Can. J. Research, F, 22 : 97-106. 1944.
10. WHITE, W. H. and COOK, W. H. Can. J. Research, D, 18 : 249-259. 1940.
11. WHITE, W. H., GIBBONS, N. E., and THISTLE, M. W. Can. J. Research. In press. 1945.
12. WHITE, W. H., GIBBONS, N. E., WOODCOCK, A. H., and COOK, W. H. Can. J. Research, D, 20 : 263-275. 1942.
13. WHITE, W. H., WOODCOCK, A. H., and GIBBONS, N. E. Can. J. Research, F, 22 : 107-118. 1944.

FURTHER BACTERIOLOGICAL STUDIES RELATING
TO EGG DRYING[3]

C. K. Johns[2] and H. L. Berard[3]

Science Service, Ottawa, Ontario

[Received for publication March 15, 1945]

In a previous publication (9) the standards and procedures employed in the bacteriological control of Canadian dried eggs for export to Britain were described, together with some of the results for 1943.   Studies conducted during the latter half of 1943 with the direct microscopic count (10) indicated that this method had considerable value in reflecting the care given the melange before drying.   On the other hand, analysis of the results obtained in testing for the presence of coliform organisms and *Escherichia coli* indicated little or no correlation with plate counts of viable organisms, direct microscopic counts, the presence of *Salmonella* species or the results of plant sanitation surveys.   The determination of coliform organisms and *E. coli* in Grade A powder was therefore dropped for 1944, a direct microscopic count of 2,000,000 per gram being substituted for it.

RESUME OF RESULTS OF 1944 OPERATIONS

As will be evident from the data in Table 1, the over-all picture as judged by the plate count of viable organisms was very satisfactory.   The improvement in average counts over 1943 paralleled improvements brought about in the drying plants following plant sanitation surveys, supplemented by routine checks conducted by the resident inspectors using the Burri slant technique (8).   With the direct microscopic counts, on the other hand, a very different picture was obtained.   While gratifyingly low during the first four months, they then rose to quite high levels, remaining so until December.   Had our analyses been confined to the plate count, we would have remained ignorant of the changed situation.

During the fourth week of May, unusually high direct microscopic counts appeared with dramatic suddenness in the powder from 3 of the 5 Western drying plants (Table 2).   In each instance the high count was due to the presence of a short, plump rod, occurring in chains of up to 10 cells, and easily mistaken for a streptococcus.   For convenience, this was referred to as the Y organism.   It was never isolated from samples of powder received at Ottawa, while attempts to isolate it from numerous samples of melange and powder at the plants soon after the outbreaks met with no success.   Plating powder on media containing sterile unheated egg, and incubating plates at temperatures ranging from 38° F. (3.3° C.) to 112° F. (44.4° C.) failed to bring about any significant increase over the standard plate count (1, 9), again suggesting that the organism did not survive the drying process.

In investigating this outbreak, samples were obtained from the start and finish of 3 days' drying at the two plants (A and C) where the Y organism first showed up, to determine whether or not any "build-up" in

count took place during the day's operations. (Previous experience had indicated the value of this practice in seeking the cause of high counts.) However, the results (Table 3) failed to indicate any consistent increase in count during the day's operations.

Because the appearance of the Y organism coincided with the change to a different batch of North's stain (1, 10), it was thought the explanation might lie in the ability of the new batch of stain to bring out this organism on stained smears, which previous batches had failed to do. However, subsequent comparative tests with old and new stains showed the Y organism to stain equally well with either, so this hypothesis had to be discarded.

The high counts due to the Y organism disappeared as suddenly as they had appeared. The organism appeared in powder made at 4 of the 5 plants in Western Canada, and was never seen in more than two carlots from any one plant. All available evidence suggested that the sporadic appearance of this organism in such large numbers could be attributed to its having invaded the contents of a small percentage of eggs; these being held at higher than average temperatures for longer periods than is customary, the organisms multiplied extensively without causing sufficient change in the appearance or odour of the eggs to enable the breaker to detect and discard them.

That high counts could be due to the eggs themselves, and not to faulty plant practice, was difficult to believe in view of the previous findings on the bacterial content of shell eggs as broken out in 2 Canadian drying plants (6, 7) supplemented by periodical counts at breaking and drying plants throughout Canada. Such findings had confirmed the conclusion reached by previous investigators that good quality shell eggs contain relatively few bacteria. However, evidence accumulated since May, 1944 has necessitated some modification of this view.

Shortly after the epidemic of high counts in the West, there was a general rise in the level of microscopic counts on powder from all over the country. This is clearly seen in Figure 1, showing the microscopic counts of consecutive carlot samples of Grade A powder. The general level of counts continued to rise until October, following which there was some decline. On the other hand, this seasonal rise was not evident in the plate counts (Figure 2 and Table 1). Since plant sanitation and practices were generally superior to those of the previous year, it seemed most unlikely that these higher levels of microscopic counts could be attributed to faulty plant operations.

The first indication that high counts might be coming from the eggs themselves was obtained in one of the Western plants at the end of May, 1944. This plant (Plant E) had had trouble with high counts for some weeks prior to the appearance of the Y organism (Table 2). Investigation revealed that, because of the extreme shortage of cold storage facilities, eggs had been broken directly out of cars, the temperature of which at times had exceeded 60° F. Melange prepared during the day was pumped into portable 80-gallon holding tanks and run into a cold room held at around the freezing point. Some of this melange would be held for up to
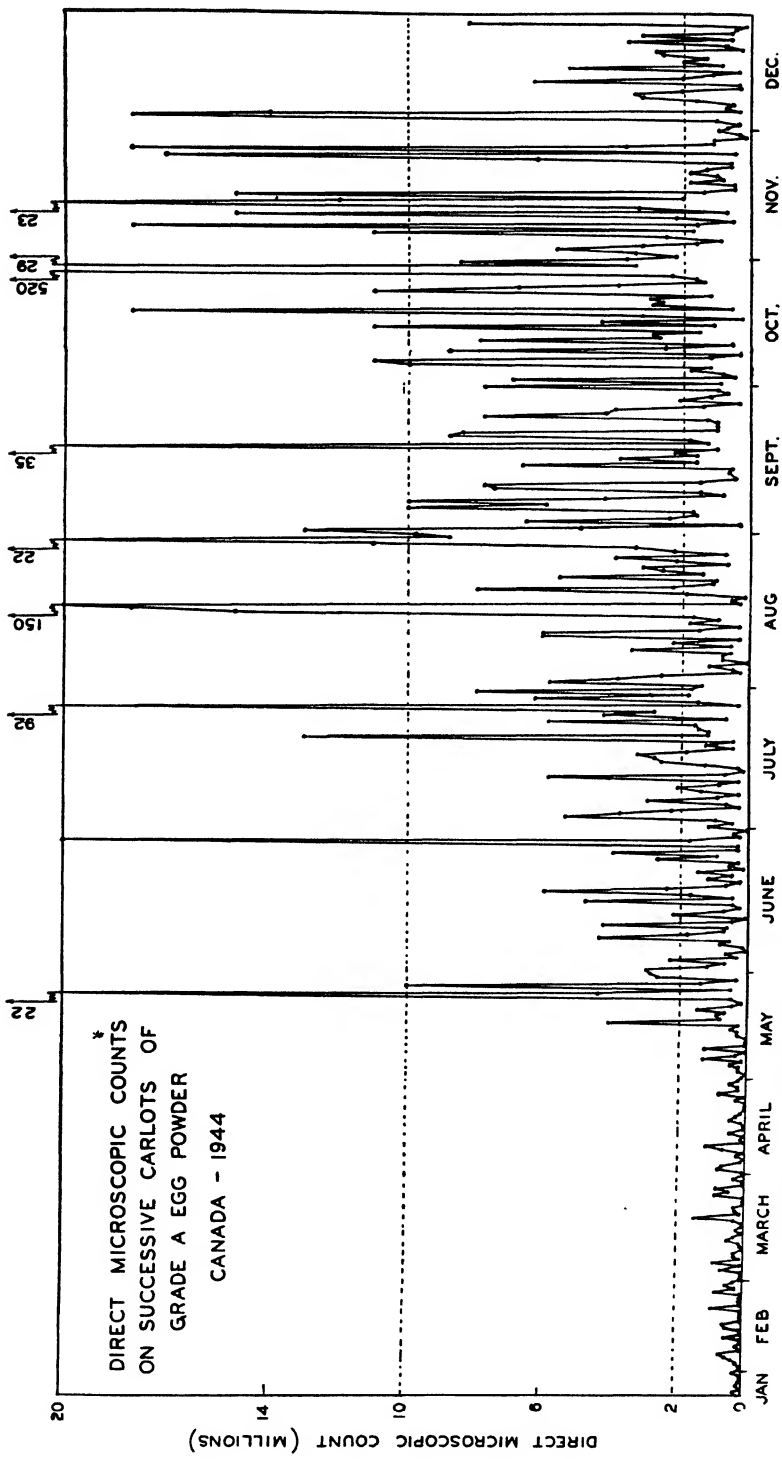
FIGURE 1. Direct microscopic counts on successive carlots of Grade A egg powder. Canada—1944.

VIABLE COUNTS ON
SUCCESSIVE CARLOTS OF
GRADE A EGG POWDER
CANADA - 1944

FIGURE 2. Viable counts on successive carlots of Grade A egg powder. Canada—1944.

16 hours before being dried.   With the slow rate of cooling of such large masses of viscous material in still air (2), considerable bacterial development could be expected.

This plant had two cars of eggs in storage; both had been "spotted" on May 15th and were held "on track" until unloaded on the 22nd.   At that time temperatures of 60° and 65° F. were recorded inside the cars. These eggs were then placed in storage at 30° F.   In order to get some idea of the bacterial content, it was decided to select 5 representative cases from each carlot, and to have these eggs broken by experienced breakers using specially sterilized equipment.   This was done on May 29th.   Six pailfuls of melange were then selected from each carlot, mixed as thoroughly as possible by beating with long handled spoons, and samples taken for analysis.   From each sample a Burri slant was prepared and incubated at room temperature for three days.   In addition, a direct microscopic smear was made using the technique of Mallmann and Churchill (12).

Because of the uneven distribution of bacteria on these smears, it was not possible to draw valid conclusions concerning the bacterial contents by microscopic examination.   The Burri slants, however, (Table 4) indicated marked variations from one pail of melange to the next.   Furthermore, it was significant that the high count slants showed practically pure cultures, in striking contrast to the heterogeneous flora obtained from swab tests on on breaking equipment (Figure 3).   This strongly suggested that the high counts were attributable to the inclusion of an occasional egg which, while normal in odour and appearance, contained enormous numbers of bacteria.
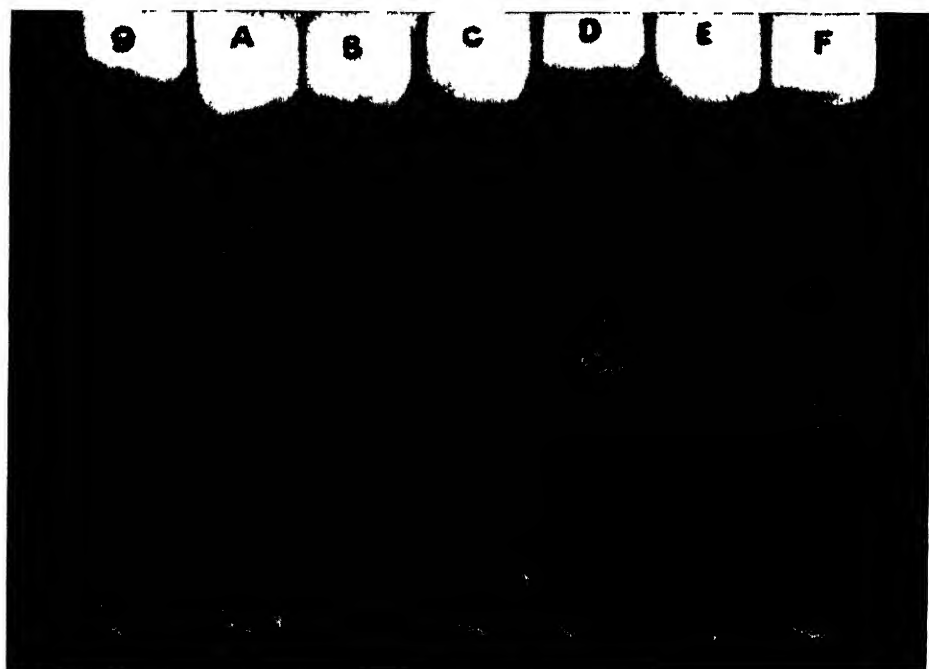
FIGURE 3.   Burri slants prepared from pails of melange (A to F) and from swabbing of a breaking cup (9).

Evidence pointing in this direction accumulated as the summer went on. Burri slant determinations made at breaking plants throughout Canada showed a much higher level of counts from May on without any evidence of a let-down in sanitation in the plants. Again, the chemist at one Ontario drying plant frequently broke out 90 to 100 eggs from a current carlot. Each egg was carefully checked for abnormality, then a Burri slant prepared. Occasionally the slant from a single apparently normal egg would be so heavily overgrown that it could not be counted, while the remainder would show few, if any, colonies. Slants made after beating the entire batch of eggs in a sterilized pail would show counts in the millions, and of the type of organism found on the slant from the high count egg. At this plant, later on in August, the writer prepared triplicate Burri slants from 30 pails of melange broken out with special precautions to reduce contamination, with the results shown in Table 5. Here again it was found that the high count slants showed practically pure cultures, strongly suggesting that a single egg was responsible for the high count on the pailful of melange.

Although a number of independent breaking plants broke and froze eggs for subsequent drying, the volume of eggs handled by the Special Products Board in 1944 was so great[4] that from early May on it was impossible to find sufficient first-class shell egg storage. Unfortunately, this coincided with the appearance of unusually warm weather, temperatures approaching or exceeding 90° F. being recorded in May from Quebec to Alberta. As a result, eggs were held for longer periods at higher temperatures than are recommended. Under these conditions, it is understandable that organisms capable of infecting and growing in the egg might multiply enormously before the egg was placed in proper cold storage. Subsequent growth would depend upon the ability of the invading organism to grow at around 30° F. The presence of a small proportion of such eggs, which could not be detected by appearance or odour, would account for the marked rise in counts noted.

EXPERIMENTS WITH ORGANISMS ISOLATED FROM HIGH COUNT EGGS OR MELANGE

In order to determine the infective ability of organisms isolated from individual eggs or carefully prepared melange, fresh eggs were obtained on several occasions through the courtesy of the Poultry Division, Central Experimental Farm. These were warmed for several hours at body temperature, then immersed for 1 to 2 minutes in cold suspensions of the organisms to be tested, after the technique devised by Haines and Moran (4) for infecting eggs without rupturing the shell. The cultures employed included species of *Achromobacter*, *Flavobacterium* and *Pseudomonas*. In the earlier experiments the eggs, after immersion in the bacterial suspensions, were held at 58 to 60° F. with a relative humidity of 50%. From 4 to 5 weeks later the eggs were broken out individually, using an ordinary egg breaking knife and cup which were washed, then treated in Roccal solution (1 : 500) for 2 minutes, after each using. The broken-out eggs were placed in sterile jars and examined for odour and appearance by members of the laboratory staff as well as by specialists from the Poultry Products Division,

---

[4] Purchases during the first 6 months of 1944 were 212% of those for the same period of 1943.

Marketing Service, Department of Agriculture.   On one occasion, 2 eggs infected with an *Achromobacter* species, and showing plate counts after 3 days at 86° F. (30° C.) of 2,500,000,000 and 2,800,000,000 per gram, respectively, had a definitely "off" odour, together with weak or stuck yolk.   All other eggs were passed as being of acceptable quality, yet counts as high as 1,400,000,000 per gram were obtained (Table 6).

In a later series of tests, fresh eggs were exposed to infection as previously described, held for 1 week at 58° F., then at 40° F. for 7 weeks before being broken out.   In this experiment, only the eggs exposed to the *Pseudomonas* cultures were found to contain significant numbers of bacteria (Table 7).   Subsequent tests on the growth range of the various cultures used in inoculation experiments showed only the *Pseudomonas* species to be capable of strong growth at 40° F. or lower.   The presence of the *Achromobacter* and *Flavobacterium* species in very large numbers in apparently normal eggs (Table 6) is strong presumptive evidence that the eggs in question had been stored at temperatures well above 40° F. for considerable periods before going into proper shell egg storage.

DETECTION OF HIGH COUNT "NORMAL" EGGS BY THEIR FLUORESCENCE

While it is now generally accepted that the majority of eggs as laid are free from micro-organisms, the possibility that eggs may carry large numbers of bacteria without any evident change in odour or appearance has been recognized (5, 14).   In the Southwestern section of the United States it is not uncommon during wet weather in early spring to find such eggs in significant numbers (13).   Since the majority of the bacteria isolated from such eggs are species of *Pseudomonas*, and since the members of this genus are known to produce substances fluorescing under ultra-violet light, the possibility of detecting such eggs at the time of breaking has been explored by some of the larger egg-breaking concerns there.   It has been reported (13) that in one plant where special ultra-violet lamps replaced the ordinary illumination in the breaking room, it was possible, by rejection of eggs showing fluorescence, to reduce the counts on melange to one-fortieth of the previous count.   While the majority of the cultures isolated in our laboratory were not *Pseudomonas* species, it seemed worth while determining the value of the ultra-violet lamp in the detection of these apparently normal high count eggs in Canada.

Through the courtesy of Swift and Company, Chicago, one of the special ultra-violet lamps from their Research Laboratories was made available for tests on storage eggs at Plant H.   An assistant, whose knowledge of egg quality was well above average, was assigned to break out the eggs.   As wide a selection as possible was obtained by picking a few eggs from each case as they were transferred to the shell egg buckets.   Each egg was broken separately into a special black enamelled cup and checked for odour, appearance and fluorescence.   If normal in odour and appearance, yet showing fluorescence, the degree of fluorescence was estimated and the egg transferred to a sterile screw-capped jar.   It was then shaken vigorously to emulsify it, and a Burri slant prepared.   The knife and cup were replaced each time a fluorescent egg or "reject" was encountered.   In order to check on the possibility of high counts from non-fluorescent eggs, 69 eggs showing no fluorescence and 19 showing doubtful fluorescence were

examined bacteriologically. Burri slants were incubated at 70° to 80° F. (21° to 27° C.) for 3 days before being counted.

The results obtained from the examination of 240 eggs are summarized in Table 8. Although there is some correlation between count and degree of fluorescence, there are many discrepancies. If an egg showing 3 + or greater is regarded as definitely fluorescent, 31.3% of such eggs had counts under 2,000, 53% under 10,000, and 75% under 40,000 per gram. Thus if all eggs showing definite fluorescence were discarded it would mean the rejection of a considerable percentage which were otherwise acceptable.

On the other hand, the data show that high count eggs do not always show fluorescence. Results obtained in the analysis of 348 storage eggs in August, 1943 (6) showed only 2.3% with counts in excess of 40,000 per gram. Taking this as the maximum acceptable count, it will be seen that in the present studies only 8 of the 28 eggs in this group showed definite fluorescence as defined above. Among the remaining 20 eggs are two with counts of more than 10,000,000 and 20,000,000 per gram, respectively. While the elimination of those eggs showing strong fluorescence would help in reducing the count, it would not prevent the occasional acceptance of eggs with very high counts, while at the same time rejecting a number of acceptable eggs.

In our studies with cultures isolated from apparently normal eggs, definite fluorescence was noted on eggs experimentally infected with *Achromobacter* and *Flavobacterium* species as well as with *Pseudomonas* when held at 58 to 60° F. (Table 6). To determine whether there was any correlation between the type of organism and the degree of fluorescence of the naturally infected egg (Table 8), cultures isolated from high count eggs were identified as to genus. The results (Table 9) indicate no definite correlation. *Achromobacter* and *Flavobacterium* species were isolated from eggs showing medium to strong fluorescence, while 1 of the 3 eggs from which a *Pseudomonas* species was isolated showed little or no fluorescence. However, the bacterial contents of the eggs infected with *Pseudomonas* species were much lower than those generally encountered where *Achromobacter* or *Flavobacterium* species were found. It is of interest to record that although the *Pseudomonas* species were the only ones showing strong growth at 29° F. (−2° C.) within 2 weeks, all but one of the other cultures showed moderate growth at this temperature after 5 weeks, while all showed good growth at 40° F. (4° C.) after 1 week.

THE PRESENCE OF STREPTOCOCCUS FAECALIS IN EGG POWDER

In view of the assertion that *Streptococcus faecalis*, an organism commonly isolated from the intestinal contents of man and other animals, is one of the commonest species found in egg powder, two representative colonies were picked from plates poured from 38 Grade A and 12 Grade B carlot samples. These included powder from each of 8 drying plants. Of 96 such cultures studied in detail, only 3 proved to be *S. faecalis*. As had been found in 1943, the commonest type of organism appearing on plates of tryptone-glucose-extract-skimmilk agar incubated for 48 hours at 37° C. (98 6° F.) was a streptococcus, so far unidentified, which forms small amounts of acid but fails to curdle litmus milk at room temperature or 30° C. (86° F.).

DISCUSSION

The experience of the past summer suggests the need for revising our opinion regarding the sanitary significance of high direct microscopic counts in dried whole egg powder.   Previously we would have been in entire agreement with the statement of Lepper, Bartram and Hillig (11) that, "In no instance did dried eggs show a microscopic count exceeding 10 million per gram . . . . . . when they were prepared from sound raw material.   In all cases where these counts were exceeded, decomposed or rotten eggs had been incorporated in the product or the eggs had been subjected to conditions after breaking-out which permitted them to sour." For at least some of the high count samples examined here in 1944, we have reason to believe that this statement would not hold true.   There was no temptation for a dryer to use sub-standard eggs, since the Special Products Board furnished graded eggs and maintained a resident inspector at the plant.   Furthermore, the dryer was faced with a stiff financial penalty if his product failed to meet the bacteriological specifications.   Consequently, dryers were very particular about the quality of eggs broken out.

Although the results obtained in 1944 indicate that some caution must be observed in interpreting high direct microscopic counts, it should not be concluded that such counts are of little value in controlling sanitation and plant practices.   As will be seen from the data in Table 10, there have been several instances in which faulty practices such as inadequate cooling of melange were not reflected in the viable count or pH value of the powder, but were detected through the direct microscopic count.   High microscopic counts due to the eggs themselves are largely, if not entirely, made up of rod forms; those due to faulty plant practices, on the other hand, are usually made up mainly of paired cocci, resembling the picture obtained in souring milk.   This distinction has often proven to be of real value when the cause of a high count is being sought.

The direct microscopic count is particularly valuable in that it does not appear to be affected by conditions of drying and subsequent storage to the same degree as is the viable count.   Plants vary greatly in the degree of bacterial destruction brought about by the drying process, as indicated by the data shown in Table 11.   Without the direct microscopic count, some plants would be credited with doing a much better job than they are actually doing, while the converse would hold true for others.   Studies intended to throw some light on the reasons for these differences in bacterial destruction between plants are under way during the 1945 drying season.

SUMMARY

As judged by plate counts, the bacteriological condition of Canadian dried eggs in 1944 was very satisfactory.

Unusually high direct microscopic counts were noted from May on. While a few of these were attributable to inadequate cooling of melange due to refrigeration failures, the majority were due to the inclusion of a small percentage of eggs which, while apparently normal in appearance and odour, yet contained enormous numbers of bacteria.   Fluorescence under ultra-violet light was of limited value in the detection of such eggs.

While a high direct microscopic count cannot always be regarded as an indication of faulty plant practice, the method can yield information in this regard which is not always obtainable through the plate count or pH value.

ACKNOWLEDGMENTS

REFERENCES

1. American Public Health Association. Standard Methods for the Analysis of Dairy Products. 8th ed. New York, N.Y. 1941.
2. Goresline, H. E. and V. H. McFarlane. Handling eggs for drying. U.S. Egg Poultry Magazine. 50 : 3; 116-119, 140-144. 1944.
3. Goresline, H. E. Private communication, November 1, 1944.
4. Haines, R. B. and T. Moran. Porosity of, and bacterial invasion through, the shell of the hen's egg. J. Hyg. 40 : 4; 453-461. 1940.
5. Jenkins, M. K. and N. Hendrickson. Accuracy in the commercial grading of opened eggs. U.S. Dept. Agr. Bull 391. 1918.
6. Johns, C. K. The bacterial content of shell eggs. Food in Canada. 3 : 12; 15-16. 1943.
7. Johns, C. K. Few bacteria in Canadian eggs. Food in Canada. 4 : 4; 16. 1944.
8. Johns, C. K. The Burri slant technique in the food industries. Food in Canada. 4 : 3; 17-18. 1944.
9. Johns, C. K. Microbiological aspects of egg powder. Sci. Agr. 24 : 8; 373-382. 1944.
10. Johns, C. K. and H. L. Berard. Direct microscopic method in bacteriological control of dried whole eggs. Food Res. 9 : 5; 396-399. 1944.
11. Lepper, H. A., M. T. Bartram and F. Hillig. Detection of decomposition in liquid, frozen and dried eggs. Jr. A.O.A.C. 27 : 204-223. 1944.
12. Mallmann, W. L. and E. Churchill. A rapid test for determining bacteria in liquid eggs. U.S. Egg Poultry Magazine. 48 : 406-407, 427-429. 1942.
13. Mink, L. D. Private communication, November 2, 1944.
14. Pennington, M. E., M. K. Jenkins, E. Q. St. John and W. C. Hicks. Bacteriological and chemical study of commercial eggs in the producing districts of the Central West. U.S. Dept. Agr. Bull. 51. 1914.

Table 1.—Monthly arithmetical averages of bacteria counts for Canadian grade A dried whole egg powder, 1943-1944

Month	Plate count (thousands per gram)		Direct microscopic count (thousands per gram) 1944	Percentage over 2,000,000
	1943	1944		
January	84	27.5	130	0
February	409	82	250	0
March	160	86	321	0
April	120	58.4	267	0
May	80	75.5	1,785	17.1
June	139	68	1,652	25.5
July	90	115	4,642	37.2
August	130	112	3,522	48.9
September	94	119	3,998	47.7
October	102	101	4,436	62.5
November	681	143	4,595	42.5
December	376	131	1,810	33.3

TABLE 2.—BACTERIA COUNTS ON CONSECUTIVE CARLOT SAMPLES OF GRADE A POWDER FROM WESTERN CANADIAN PLANTS

Plant	Date analysed	Count per gram	
		Direct microscopic	Plate
A H–18	April 22	< 70,000	17,000
J– 2	May 3	< 70,000	9,800
J–12	May 13	< 70,000	12,000
J–19	May 24	22,000,000 Y	28,000
J–30	June 3	340,000	22,000
B H–10	April 15	70,000	18,000
H–24	May 1	150,000	9,500
J–12	May 13	290,000	10,000
J–24	May 29	2,700,000	4,900
K– 9	June 15	2,200,000	7,100
K–17	June 17	6 000,000 Y	36,000
K–23	June 23	590,000	12,000
C H–30	May 4	220,000	83,000
J– 5	May 8	1,300,000	44,000
J–10	May 15	510,000	45,000
J–18	May 25	4,400,000 Y	25,000
J–25	May 23	440,000	33,000
J–30	June 2	660,000	13,000
K– 5	June 7	70,000	37,000
D J– 1	May 3	220,000	6,800
J– 4	May 9	< 70,000	8,100
J–10	May 12	< 70,000	11,000
J–14	May 16	810,000	7,900
J–17	May 19	660,000	8,700
J–22	May 24	510,000	10,000
J–23	May 27	10,000,000 Y	2,300
J–26	May 27	340,000	5,300
E H–20	April 26	810,000	220,000
J– 4	May 9	1,300,000	530,000
J–15	May 16	4,100,000	740,000
J–27	May 31	2,700,000	37,000
K– 6	June 7	810,000	25,000
K–20	June 29	220,000	65,000

Y = high count due to Y organism.

TABLE 3.—BACTERIA COUNTS ON SPECIAL SAMPLES OF GRADE A POWDER FROM TWO WESTERN CANADIAN PLANTS, 1944

Plant	Time of sampling	Direct microscopic count	Plate count
	Start of run, May 25	19,000,000 Y	8,600
	Finish of run, May 25	23,000,000 Y	23,000
A	Start of run, May 26	18,000,000 Y	9,100
	Finish of run, May 26	140,000,000 Y	83,000
	Start of run, May 27	17,000 000 Y	10,000
	Finish of run, May 27	7,300,000 Y	88,000
	Start of run, May 26	17,000 000 Y	8,300
	Finish of run, May 26	6,600,000 Y	38,000
C	Start of run, May 27	21,000,000 Y	20,000
	Finish of run, May 27	8,400,000 Y	20,000
	Start of run, May 28	7,000,000 Y	10,000
	Finish of run, May 28	12,000,000 Y	11,000

Y = high count due to Y organism.
Average length of run = 21 hours.

TABLE 4.—COUNTS OBTAINED BY BURRI SLANT METHOD ON PAILS OF
MELANGE BROKEN OUT WITH SPECIAL PRECAUTIONS.
PLANT E, MAY 29, 1944

Lot No.	Pail No.	Count per gram
2529	1	1,400,000
	2	< 2,000
	3	4,000
	4	10,000
	5	20,000
	6	320,000
2536	1	300,000
	2	22,000
	3	58,000
	4	10,000
	5	600,000
	6	4,000

TABLE 5.—COUNTS OBTAINED BY BURRI SLANT METHOD ON PAILS OF MELANGE BROKEN
OUT WITH SPECIAL PRECAUTIONS. PLANT H, AUGUST 23-24, 1944

Lot No.	2172	3204	2167	2167	2167
Time of sampling	23rd a.m.	23rd p.m.	24th a.m.	24th a.m.	24th a.m.
Pail No.	Count per gram				
1	2,000	3,700,000	800,000	8,000	2,000
2	1,000	3,200,000	1,100,000	2,400,000	3,800,000
3	1,600,000	1,400,000	7,000	900,000	1,600,000
4	1,500,000	4,000,000	2,000,000	3,000,000	4,000
5	1,000	1,100,000	4,000,000	3,800,000	1,200,000
6	1,300,000	1,200,000	16,000	700,000	46,000

TABLE 6.—RESULTS OF EXAMINATION OF EGGS EXPOSED TO INFECTION WITH CULTURES OF
BACTERIA ISOLATED FROM APPARENTLY NORMAL EGGS

Culture	Species	Storage conditions	Fluorescence	Plate count per gram
A	*Achromobacter*	4 weeks, 58-60° F.	+++	2,500,000,000
B 1	*Flavobacterium*	4 weeks, 58-60° F.	?	300,000,000
E	*Pseudomonas*	4 weeks, 58-60° F.	?	120,000
K	*Pseudomonas*	4 weeks, 58-60° F.	++++	260,000,000
N	*Achromobacter*	4 weeks, 58-60° F.	?	160,000,000
A	*Achromobacter*	5 weeks, 58-60° F.	?	2,800,000,000
B 1	*Flavobacterium*	5 weeks, 58-60° F.	+++	86,000,000
J	*Flavobacterium*	5 weeks, 58-60° F.	?	800,000,000
K 5	*Flavobacterium*	5 weeks, 58-60° F.	?	1,400,000,000
R	*Achromobacter*	5 weeks, 58-60° F.	++	84,000,000

TABLE 7.—RESULTS OF EXAMINATION OF EGGS EXPOSED TO INFECTION WITH CULTURES OF
BACTERIA ISOLATED FROM APPARENTLY NORMAL EGGS*

Culture	Species	Appearance and odour	Fluorescence	Bacteria per gram
B 1 a	*Flavobacterium*	O.K.	—	8,000
b	*Flavobacterium*	O.K.	—	< 2,000
E a	*Pseudomonas*	White sl. green	+++++	720,000,000
b	*Pseudomonas*	O.K.	—	< 2,000
G a	*Pseudomonas*	White sl. green	++++	310,000,000
b	*Pseudomonas*	White sl. green	++++	430,000
J a	*Achromobacter*	O.K.	—	< 2,000
b	*Achromobacter*	O.K.	—	< 2,000
K a	*Pseudomonas*	White sl. green	++++	480,000,000
b	*Pseudomonas*	White sl. green	++++	120,000
N a	*Achromobacter*	O.K.	—	< 2,000
b	*Achromobacter*	O.K.	—	< 2,000
R a	*Achromobacter*	O.K.	—	< 2,000
b	*Achromobacter*	O.K.	—	< 2,000
Control a	—	O.K.	—	< 2,000
b	—	O.K.	—	< 2,000

* Eggs held for 1 week at 58° F., then for 7 weeks at 40° F. before being broken out.

TABLE 8.—CORRELATION BETWEEN FLUORESCENCE AND BACTERIAL CONTENT OF
STORAGE EGGS BROKEN OUT NOVEMBER 24, 1944

Bacteria count per gram	No. of eggs	Degree of fluorescence						
		−	+?	+	++	+++	++++	+++++
< 2,000	120	34	8	45	23	9	1	
2,000 − 10,000	69	22	4	19	17	7		
11,000 − 50,000	25	8	3	1	5	7		1
51,000 − 200,000	14	6	3	2	3			
201,000 − 1,000,000	5		1		2		2	
1,010,000 − 5,000,000	1						1	
>5,000,000	6			1	1	2	2	
	240	70	19	68	51	25	6	1

TABLE 9.—RELATIONSHIP BETWEEN BACTERIA COUNT AND FLUORESCENCE OF APPARENTLY NORMAL EGGS AND THE CHARACTERISTICS OF THE ORGANISMS ISOLATED THEREFROM

Culture No.	Genus	Growth −2° C. in 2 weeks	Bacteria count per gram	Degree of fluorescence of	
				Egg*	Culture†
T 1	*Achromobacter*	—	>20,000,000	++	—
T 2	*Achromobacter*	—	>10,000,000	+	—
T 3	*Achromobacter*	—	280,000	?	—
T 12	*Achromobacter*	—	>10,000,000	+++++	—
T 18	*Achromobacter*	—	9,000,000	+++++	—
T 6	*Flavobacterium*	+	8,000,000	+++	—
T 123	*Flavobacterium*	—	10,000 000	+++	—
T 10	*Pseudomonas*	+++	450,000	+++++	+++
T 97	*Pseudomonas*	+++	66,000	?	+++
T 116	*Pseudomonas*	+++	240,000	+++++	+++

* Determined by U.-V. light at the moment of breaking.
† Determined by U.-V. light on 24-hour growth in Georgia & Poe's asparagine medium.

TABLE 10.—DATA ON SAMPLES WHERE HIGH COUNTS WERE BELIEVED TO BE DUE TO FAULTY PLANT PRACTICES

Plant	Carlot No.	pH Value	Plate count	Direct microscopic count
B	L–20	8.68	100,000	13,000,000
	L–25	8.65	240,000	92,000,000
C	K–26	8.70	100,000	20,000,000
	M–30	8.72	230,000	13,000,000
	O–25	8.54	2,000,000	520,000,000
F	M–17	7.82	64,000	150,000,000
	N–14	8.57	29,000	35,000,000
	P–24	8.58	39,000	18,000,000
G	M–14	8.67	790,000	15,000,000
	O–14	8.54	260,000	11,000,000
	O–20	8.61	500,000	18,000,000

TABLE 11.—EFFICIENCY OF BACTERIAL DESTRUCTION AS JUDGED BY RATIOS OF PLATE COUNTS TO DIRECT MICROSCOPIC COUNTS ON DRIED WHOLE EGGS

(October-December, 1944)

Plant	Carlot No.	Plate count per gram	Direct microscopic count per gram	Ratio
	O– 9	390,000	2,900,000	1 : 7.4
	O–23	26,000	1,400,000	1 : 53.8
	O–31	380,000	2,200,000	1 : 5.8
	P– 9	1,000,000	2,000,000	1 : 2.0
E	P–17	590,000	1,800,000	1 : 3.1
	P–25	340,000	1,100,000	1 : 3.2
	Q– 5	330,000	510,000	1 : 1.5
	Q–14	150,000	810,000	1 : 5.4
	Q–21	110,000	440,000	1 : 4.0
			Average	1 : 9.6
	O– 9	27,000	7,900,000	1 : 292.6
	O–22	12,000	11,000,000	1 : 916.6
	O–30	13,000	5,700,000	1 : 438.5
	P– 9	24,000	12,000,000	1 : 500.0
F	P–18	20,000	17,000,000	1 : 850.0
	P–24	39,000	18,000,000	1 : 461.5
	Q– 5	20,000	3,400,000	1 : 170.0
	Q–18	18,000	3,200,000	1 : 177.7
	Q–26	50,000	8,200,000	1 : 164.0
			Average	1 : 441.2

REDUCTION OF SPATIAL TEMPERATURE VARIATIONS IN AIR-COOLED COLD STORAGE ROOMS. I.[1]

By T. A. Steeves[2] and W. H. Cook[3]

Abstract

The presence of boxes was found to increase the spatial temperature variations in a refrigerated room. By forcing the air to pass through the stack, e.g., by blocking the aisles, such variations were reduced to approximately the value prevailing in the empty room. Dunnage spacing also reduced temperature variations, but no significant difference was found between spacings varied from 5/16 in. to 2 in.

Introduction

Uniform temperatures are recognized as essential for the satisfactory storage of perishable goods in cold stores. In practice, temperature variations in time and space are common to most commercial warehouse rooms. Observations made in such rooms show that the time variations are seldom less than 1° F., and superimposed on this are the spatial variations, which are usually of greater magnitude. In fact, spatial variations of 2° F. are met frequently and still larger variations are not uncommon.

Temperature fluctuations in time are usually associated with: small storage rooms having low thermal capacity; automatic cooling equipment of an on-and-off type; faulty operation or equipment. Such variations are readily detected with a single thermometer, and since remedial measures are comparatively well understood they can generally be reduced to a reasonable level.

In contrast, spatial temperature variations are more difficult to detect and control. With only one or two thermometers placed at accessible positions in the room, many operators will deny the existence of such variations. Even if they are observed there is little that can be done to improve the situation, short of restacking and possibly redesigning the entire room. The present study was undertaken to determine the factors responsible for these spatial variations and to reduce them if possible by improved methods of air circulation.

Forced air cooling systems of modern design generally produce more uniform temperatures than the older type of convection-cooled rooms. Systems comparable with those used in air-conditioning applications usually yield satisfactory temperature uniformity in empty warehouse rooms. When the room is filled, however, the stack of products obstructs air movement and variations of detrimental magnitude frequently become evident even in the aisles. If

the stored product is exothermic, e.g., apples, these temperature variations are enhanced further as the temperature of the air in the interior of the stack is usually higher than that of the air in the aisles.

The spatial temperature variations in a room cooled by forced air can be divided into two portions: first, a systematic increase in air temperature in the direction of flow resulting from heat absorption from the walls and product; second, irregular variations and trends resulting presumably from local peculiarities of cooling or stacking arrangements. The magnitude of the first variation is a function of the heat load and air flow, and can be reduced to any desired level in designing the room or equipment. Little is known about the factors affecting the second class of spatial variation and it is with these that the present studies are concerned.

The most generally applicable solution to the problem appears to be the development of a method that would force the air to pass through the entire width of the stack of products. This condition can best be achieved by blocking all air channels outside the stack parallel to the direction of air movement. The contents of the stack should be spaced so as to distribute the air uniformly across it, without unduly resisting the air flow. Investigations on product spacing have been made in South Africa (1, 2) in connection with the precooling of fruit. Close stacking combined with suitable baffles across open spaces resulted in higher rates of cooling, but the temperature gradients were generally higher than in "open" stacks. These results are scarcely applicable to the present problem since precooling represents a highly "unsteady" state compared with the maintenance of the desired storage temperature. British investigations (3, 4) have shown that more uniform temperature conditions are obtained during storage periods if the conventional dunnage or spacing strips are avoided.

Methods

These tests were made in an experimental room of about 700 cu. ft. capacity provided with bottom-to-top air circulation. The cool air entered through a slatted false floor and escaped through a single central port near the ceiling. Tests were made first on the empty room, then after introducing a false load of empty boxes to obstruct the air flow, and finally after introducing known heat loads by means of electrical heaters placed centrally in the first layer of boxes.

The object of adding heat was to exaggerate the spatial temperature variations and thus permit a more adequate study of the other factors under investigation rather than to study *per se* the effect of added heat. The effect of different heat loads could only be investigated under conditions that permit the control or evaluation of the portion of the temperature variations attributable to the temperature gradient in the air resulting from heat absorption. These factors had to be held over for later study. While the temperature gradient due to heat absorption by the air was included in the spatial variations as described here, the experimental conditions were chosen to minimize

the magnitude of this gradient. The room was well insulated, jacketed on the top, bottom and one side by spaces at equal or lower temperatures. A comparatively high air flow was used and the air travel through the stack was in the short vertical direction.

Effective means for blocking the vertical voids outside the stack presented a special problem. Preliminary studies showed that obstructions such as canvas dams were only partly effective since the air was merely diverted at the barrier and returned to the path of least resistance. The voids were, however, effectively closed by inflating latex-coated shelter duck bags, of suitable size, in the open spaces. In practice a series of these bags connected with hose, was inflated by means of a small auxiliary fan maintaining an air pressure of 3 to 4 in. water. The bags were readily collapsed or inflated by starting or stopping the fan, thus eliminating the need for valves.

The false load consisted of six layers of 24 boxes (1 × 1 × 2 ft.) per layer. Spacing strips were provided between each horizontal layer and also between each pair of vertical piles. Spacings of 2, 7/8, 5/16, and 0 in. were tested, with the surrounding vertical voids both open and closed in turn and at several levels of added heat.

The value of each experimental arrangement was assessed from observations indicating the uniformity of temperature and air flow. Measurements of air flow through the stack were made with the hot wire anemometer described previously (5). This measurement was, however, of limited applicability since the majority of the spacings chosen for study were too small to accommodate the instrument. In general, measurements could be made only above the stack and these were subject to some uncertainty since the spacings were narrower than the sensitive portion of the instrument.

The spatial temperature variations were estimated from temperature readings taken at 14 positions within the stack. The position of the thermocouples and the position of the boxes containing the heating elements are shown in Fig. 1. In addition the temperature of the air entering and leaving the room was measured, but these observations were not included in the computations. The temperature at each test position was recorded hourly on a carefully standardized recording instrument, and a single test usually extended over a 16-hr. period or longer.

The observed variations in such a series of temperature measurements include those attributable to spatial variations, fluctuations with time, and experimental errors. Every precaution was taken to maintain a constant temperature throughout the test period in order to minimize the fluctuations with time. The results of tests showing appreciable time variations were discarded. Nevertheless it was usually possible to demonstrate small but statistically significant differences between observations taken at different times for tests that were considered satisfactory. Statistical methods were therefore employed to eliminate the effect of these time variations, to obtain the standard deviation attributable to spatial variation for each test condition,
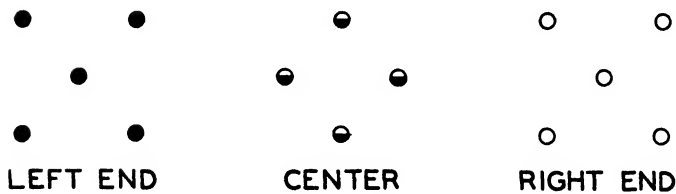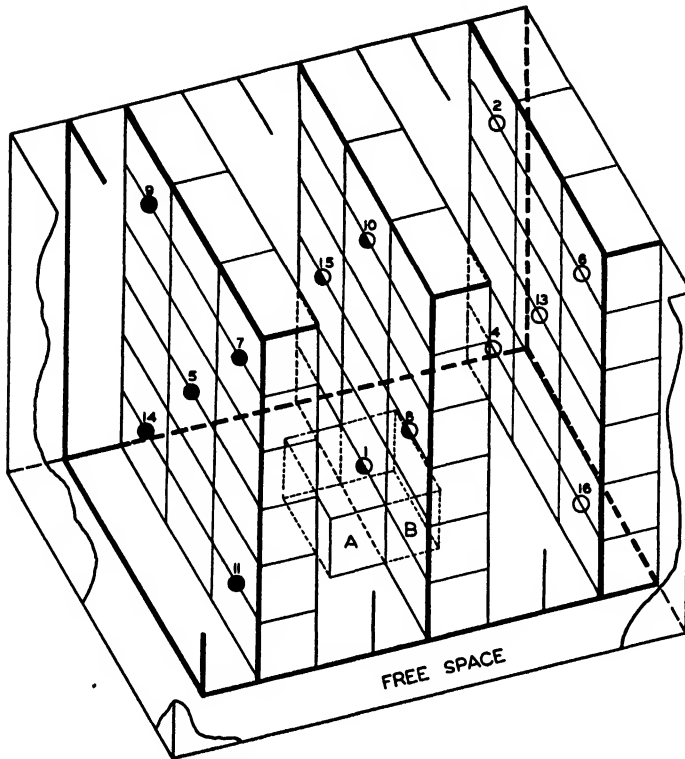
FIG. 1. *Arrangement of thermocouples and boxes in test room.   Numbered points indicate position of thermocouples.   Boxes A and B only contained heating elements.*

and to test the significance of this spatial variation by comparison with the experimental error.

When the series of experiments was complete, the standard deviations of the mean temperature, attributable to spatial variations, were themselves subjected to an analysis of variance to determine the effect of the several conditions and arrangements under test.

Results of Air Distribution Measurements

The first series of air velocity measurements was made over piles having 2-in. spacings and composed of two to five layers of boxes.   In making these observations three distinct regions could be observed in the horizontal cross-

section of the room; first, the aisles or voids which were left open in these tests; second, the boxes adjacent to the aisles; and third, the interior of the stack. Measurements were made at 60 points in each of these three regions and the results grouped in this way for analysis. The limits of Regions 2 and 3 were necessarily defined arbitrarily, and were taken as being of equal area.

The average velocity for each condition tested is shown in Table I. The velocity was highest in the interior of the stack, intermediate in the region adjacent to the aisles, and least in the aisles proper. The analysis of variance shows that the observed differences were statistically significant. The addition of successive layers of boxes tended to reduce the velocity in the interior of the stack, and had a similar but smaller effect on the region adjacent to the aisles and practically no effect in the aisles themselves.

By measuring the open area available for air movement in each region described in Table I it was possible to compute the air flow for each region and for the room as a whole. Such figures are subject to some uncertainty, for in addition to the errors of estimating the air velocity and the open area involved there is also an arbitrary element introduced in definition of the borders of each region. Nevertheless the figures so obtained appear to be informative. The results showed that the regions of highest velocity actually

TABLE I

EFFECT OF PARTIAL LOADING ON AIR VELOCITY IN DIFFERENT REGIONS

Dunnage spacing, in.	Layers of boxes	Measured volume of air in circulation, c.f.m.	Horizontal region above stack		
			Vert. voids or aisles, ft./min.	Adjacent to aisles, ft./min.	Interior, ft./min.
2	2	785	21	42	63
2	3	750	20	37	55
2	4	740	19	37	47
2	5	710	18	35	41

ANALYSIS OF VARIANCE

Source of variance	Degrees of freedom	Mean square
"Areas"	2	6875**
Residual A† "error"	15	608
Layers	3	256**
Areas × layers	6	64
Residual B "error"	45	28

** *Exceeds 1% level of significance.*
  † *Since the general means for the three areas were determined at a different level of precision than those for layers, the larger error "A" was used for assessing the significance of differences among areas.*

received the least air, with about 45% of the total air flow passing through the aisles under all conditions. The rate of air circulation computed from the area–velocity measurements for the room as a whole accounted for about 70% of the actual air flow, as measured by other means and reported in Table I.

Since these results indicated that a large portion of the air passed through the aisles, a second series of measurements was made with the aisles both open and closed. The stack was six boxes high, and both 2 in. and 7/8 in. spacings were used. The results obtained appear in Table II. The absolute values in this table are not strictly comparable with those in Table I as some alter-

TABLE II

EFFECT OF DUNNAGE SPACING AND CLOSING AISLES ON AIR VELOCITY IN DIFFERENT REGIONS
OF FULLY LOADED ROOM

Dunnage spacing, in.	Measured volume of air in circulation, c.f.m.	Horizontal region above stack		
		Vertical voids or aisles, ft./min.	Adjacent to aisles, ft./min.	Interior, ft./min.
2	760	19	34	60
2	750	Closed	62	61
7/8	700	30	34	42
7/8	670	Closed	64	62

ANALYSIS OF VARIANCE

Source of variance	Degrees of freedom	Mean square
Aisles open		
Areas (above stack)	2	2089***
Residual A† "error"	15	126
Dunnage spacing	1	50
Areas × dunnage	2	190
Residual B "error"	15	149
Aisles closed		
Areas (above stack)	1	16
Residual A† "error"	10	50
Dunnage spacing	1	11
Areas × dunnage	1	2
Residual B "error"	10	37

*** *Exceeds 0.1% level of significance.*
    † *Since the general means for the three areas were determined at a different level of precision than those for layers, the larger error "A" was used for assessing the significance of differences among areas.*

ations were made in the equipment and stacking arrangements between these two experiments.   Nevertheless with the aisles open the air velocities behaved in a manner similar to that observed in the previous experiment.   When the aisles were closed the velocities in the two regions of the stack were essentially the same.   The smaller spacing did not appear to increase the velocity but this may be attributable to the difficulty of measuring the velocity in a narrow space with the available anemometer.

As indicated earlier, the conversion of these velocity figures to air flow is subject to considerable error and the details are therefore not presented. These calculations showed, however, that about twice as much air passed through the aisles, when these were open, with 7/8 in. spacing as compared with 2 in. spacing in the stack.   The total air flow, measured by independent means, and reported in Tables II and III shows the extent to which the closing of the aisles and the use of smaller spacing acted to reduce the air flow.

Results of Temperature Distribution Measurements

The statistical treatment of the results, described earlier, permitted the spatial variations observed for each condition or arrangement to be described as a standard deviation, independent of fluctuations in time.   These values for each condition in which heat was not added appear in Table III.   The

TABLE III

AVERAGE SPATIAL TEMPERATURE VARIATIONS OBSERVED UNDER VARIOUS CONDITIONS WITHOUT ADDED HEAT

Mean stack temperature, °F.	Mean air flow, c.f.m.	Vertical voids or aisles	Dunnage spacing, in.	Spatial variations as standard deviation, °F.
26	770	Empty room		0.21
34	760	Open	2	0.63
28	750	Closed	2	0.65
35	700	Open	7/8	0.50
37	670	Closed	7/8	0.48
37	675	Open	5/16	0 41
30	635	Closed	5/16	0 24
30	625	Open	0	0 97
36	500	Closed	0	0 49

complete results, including the effect of adding heat at three levels, are shown graphically in Fig. 2.   Reference to Fig. 1 shows that one of the 14 thermocouples (No. 1) in the stack was adjacent to the boxes containing the heaters. Inclusion of the values obtained at this point greatly exaggerated the estimate of the spatial variations when heat was added.   Since the relative results were the same this value was excluded from the computations.
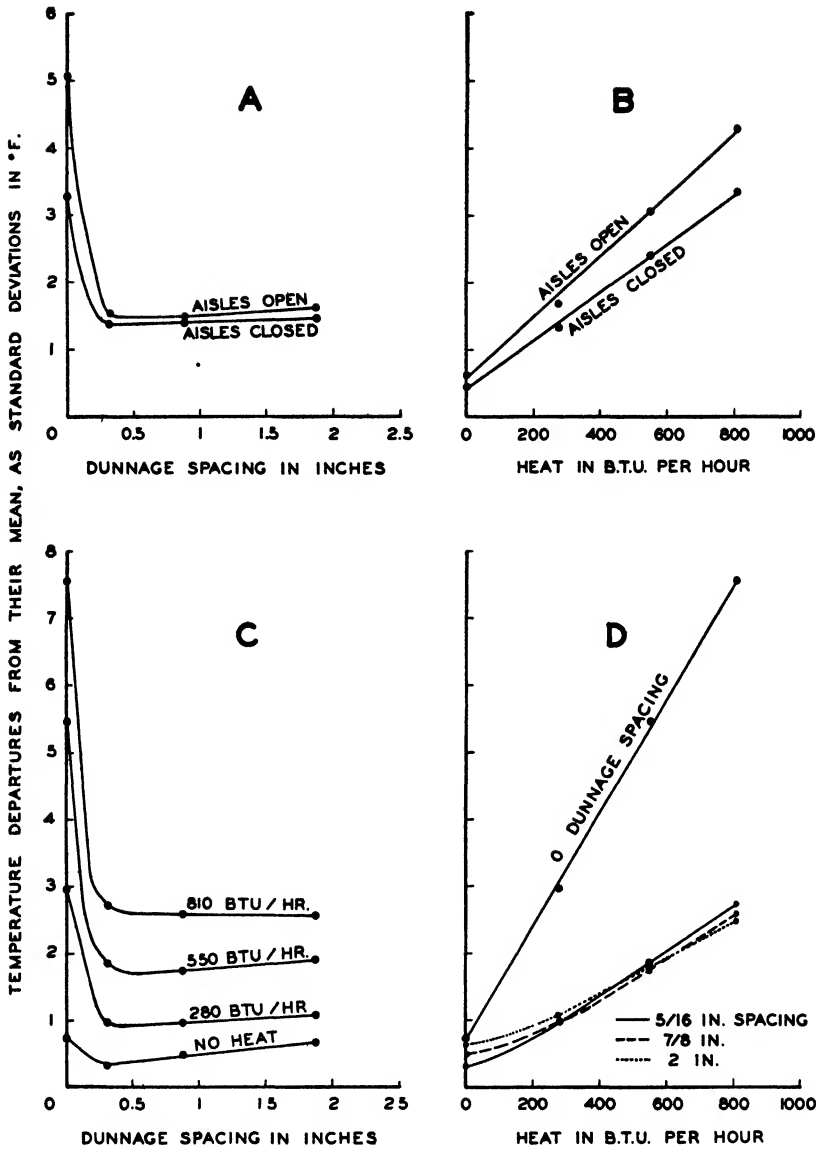
FIG. 2.   *Effect of heat, dunnage and open aisles on spatial temperature variations.*

Reference to Table III shows that the spatial temperature variation in the empty room, expressed as a standard deviation, was 0.21° F.   The introduction of the false load of boxes, but without added heat, caused a threefold increase to 0.63° F.   This increase must be attributed entirely to the obstruction of air movement.   The closest approach to the minimum deviation observed in the empty room was the value of 0.24° F. obtained at 5/16 in. spacing with the aisles closed.   The results as a whole show that the spacing must be reduced to obtain sufficient resistance to distribute the air through

the stack, and under these conditions the voids must be closed in order to force the air to traverse the pile. One complicating factor was the gradual reduction in air flow as the resistance increased. While this may have tended to increase the spatial variations by increasing the temperature gradient due to heat absorption it in no way invalidates the results. Closing the aisles was the main factor causing a reduction in air flow, but this practice actually halved the temperature variations. Had it been possible to maintain the higher air flow, the effect of closing the aisles might have been even more favourable.

The detailed results of the entire experiment are plotted in Fig. 2 and the results of an analysis of variance are given in Table IV. While this analysis shows that dunnage spacing had a significant effect on the temperature variations (Fig. 2A), a further breakdown shows that this lies entirely between dunnage versus no dunnage, and not between the individual spacings used.

TABLE IV

ANALYSIS OF VARIANCE OF SPATIAL TEMPERATURE VARIATIONS AS AFFECTED BY ALL FACTORS STUDIED

Source	Degrees of freedom	Mean square
Dunnage	3†	
Dunnage vs. no dunnage	1	.43 48**
Spacings	2	
		0.02
Aisles (open vs. closed)	1	
		2.25**
Heat	3	
		16.64**
Dunnage × aisles	3†	
Dunnage vs. no dunnage × aisles	1	
Spacings × aisles	2	4 21**
		.01
Dunnage × heat	9†	
Dunnage vs. no dunnage × heat	3	
Spacings × heat	6	6 26**
		0.02
Aisles × heat	3	
		0.24
Residual	9	0.14

** *Exceeds 1% level of significance.*
  † *The degrees of freedom attributable to dunnage have been partitioned as shown to illustrate that the entire significance lies between dunnage vs. no dunnage.*

The analysis of variance shows that the closing of the aisles reduced the spatial temperature variations significantly at all heat levels (Fig. 2B). Added heat causes a linear increase in the standard deviations which is highly significant statistically. As previously indicated, this may be due largely to an increase in the systematic temperature gradient at the higher heat loads. The remaining significant interactions in Table IV and Fig. 2D again show that the presence or absence of dunnage is responsible for the differential behaviour noted, rather than the dunnage spacings as such.

Summary and Conclusions

The limitations of these experiments must be recognized at the outset: the room was small compared with those used in practice; the effect of air flow was not studied although it varied somewhat according to the type of test; and the system of air circulation used was bottom-to-top and not the side-to-side or end-to-end systems more commonly used in large warehouses. Further experiments are being conducted in an effort to resolve some of these factors and attempt to assess the general applicability of the results.

In spite of these limitations a few facts have been established. The introduction of a stack of product, even if it is not exothermic, increases the spatial temperature variations compared with those observed in the empty room. It follows that the observation of satisfactorily uniform temperatures in an empty room is no indication that detrimental spatial variations will not occur when the room is filled. While the temperature variations increase with increasing heat loads, as might be expected, the experiments were inadequate on this point. The effect of additional heat can only be studied under conditions that permit control or evaluation of the gradients due to heat absorption.

The air velocity measurements show substantial difference in air movement, both within the stack, and between the stack and the aisles. The indications are that in spite of a uniform initial distribution through a false floor nearly half the air moves through the aisles rather than through the stack. Closing the aisles with inflated bags rendered the air flow through the stack more uniform and reduced the spatial temperature variations. With respect to dunnage, the temperature variations increased significantly when dunnage was omitted. No significant differences in the spatial variations were observed with different dunnage spacing ranging from 5/16 to 2 in.

References

1. DAVIS, R. Report of the Low Temperature Research Laboratory, Capetown, for the year 1933: 11-30. Department of Agriculture and Forestry, Pretoria, Union of South Africa.
2. DAVIS, R., DREOSTI, G. M., and OTTLE, K. A. Report of the Low Temperature Research Laboratory, Capetown, for the year 1934–35; 29-60. Department of Agriculture and Forestry, Pretoria, Union of South Africa.
3. HARDY, J. K. Report of the Food Investigations Board for the year 1937 : 233-258. H.M. Stationery Office, London, England.
4. SMITH, A. J. M. Report of the Food Investigations Board for the year 1934 : 235-239. H.M. Stationery Office, London, England.
5. STEEVES, T. A., CHADDERTON, A. E., and COOK, W. H. Can. J. Research, F, 23 : 192-197. 1945.

DRIED WHOLE EGG POWDER

XVII. OBJECTIVE TESTS AND BAKING QUALITY[1]

By Margaret Reid[2] and Jesse A. Pearce[2]

Abstract

Potassium chloride value, refractometric value, the fluorescence of defatted egg powder in 10% potassium chloride, and the fluorescence of untreated dried material correlated equally well with the foaming volume of a mixture of dried egg, sugar, and water, and with the loaf volume of the sponge cake prepared from the powder ($|r|$ values from .81 to .90). A significantly closer relationship was observed between foaming volume and loaf volume ($r = .96$). Since foaming volume was more precise and easier to determine than baking volume, it was concluded that foaming volume was a more desirable test of baking quality.

Introduction

Previous work in these laboratories on objective methods for measuring the quality of egg powders (5) has been confined to assessing their palatability. Since the use of dried egg for baking is as important as its use in the preparation of egg dishes and since the estimation of baking quality by loaf volume measurements is cumbersome, it was considered advisable to study the relationship of some of these objective tests to baking quality.

Materials and Methods

Nine powders from the main chamber and secondary collectors of plants currently producing dried egg in Canada and four powders prepared in a laboratory spray drier (8) were used in this experiment. The powders ranged in quality from excellent to inedible.

The objective tests used included: solubility in 10% potassium chloride (5), the refractive index of the defatted material in 5% sodium chloride (6), the fluorescence of the defatted powder in 10% potassium chloride (4), the fluorescence of untreated dried egg (2), and foaming volume (1). In addition an index of soluble protein materials was obtained by evaluating the nitrogenous material in the filtrate used to determine potassium chloride solubility (Kjeldahl). This was recorded as the percentage of protein dissolved, calculated on the weight of sample taken. Results from the above tests were compared with volumes of sponge cakes (7) as the criterion of baking quality.

Procedures for determining foaming volume and loaf volume varied somewhat from those previously described. Duplicate determinations of foaming volume were made using 19 gm. of egg powder, 60 gm. of sugar, and 56 ml. of distilled water. The sugar and egg powder were mixed and the water added gradually to form a homogeneous mixture. This mixture, after being warmed

---

to 40° C. (104° F.) in a water-bath, was removed to a cabinet, regulated at 40° C. and a relative humidity of 80%, and beaten for 10 min. in a 'Mixmaster' set at number 10 speed. The resulting foam was then transferred to a graduate and its volume measured.

Ingredients for loaf volume determinations were 12 gm. of dried egg, 40 gm. of sucrose, 40 gm. of unsifted flour, and 38 ml. of distilled water. After blending the sugar and egg in a bowl, 5 ml. of water was added to make a paste, which was then formed into a homogeneous fluid with the remainder of the water. This mix was allowed to stand in the high humidity cabinet for 30 min., then removed to the baking laboratory and beaten for 10 min. at number 10 speed on a Mixmaster under laboratory conditions of 27° C. (80° F.) and 65% relative humidity. The flour was added with the least possible mixing otherwise the ultimate loaf volume was considerably reduced. The batter was transferred rapidly to a baking tin (10 × 5 × 5 cm.) and baked for 40 min. at 170° C. (338° F.). Four cakes were made from each sample of powder and the volumes measured (3).

Results

The correlations between objective tests and loaf volume are given in Table I and shown graphically in Figs. 1 and 2. All correlations were highly significant. The soluble nitrogen was the least satisfactory of the measure-

TABLE I

CORRELATIONS OF QUALITY TESTS WITH FOAMING VOLUME AND LOAF VOLUME

Quality test	Correlation with	
	Foaming volume	Loaf volume
Soluble protein index	.79**	.78**
Potassium chloride value	90**	.88**
Refractometric value	85**	89**
Fluorescence of potassium chloride extract	− 88**	− 85**
Fluorescence of powder	− 87**	− 81**
Foaming value	—	96**

**Indicates 1% level of statistical significance.

ments for predicting baking quality. The potassium chloride value, refractometric value, the fluorescence of a 10% potassium chloride extract of defatted egg powder, and the fluorescence of untreated dried egg were more satisfactory and about equally effective as methods of predicting loaf volume. Foaming volume was the most satisfactory method for predicting baking quality ($r = .96$). This correlation coefficient was significantly higher than all other coefficients obtained.

In spite of the high correlation, foaming volume could be used to predict loaf volume only within ± 30 ml. However, this lack of accuracy may be
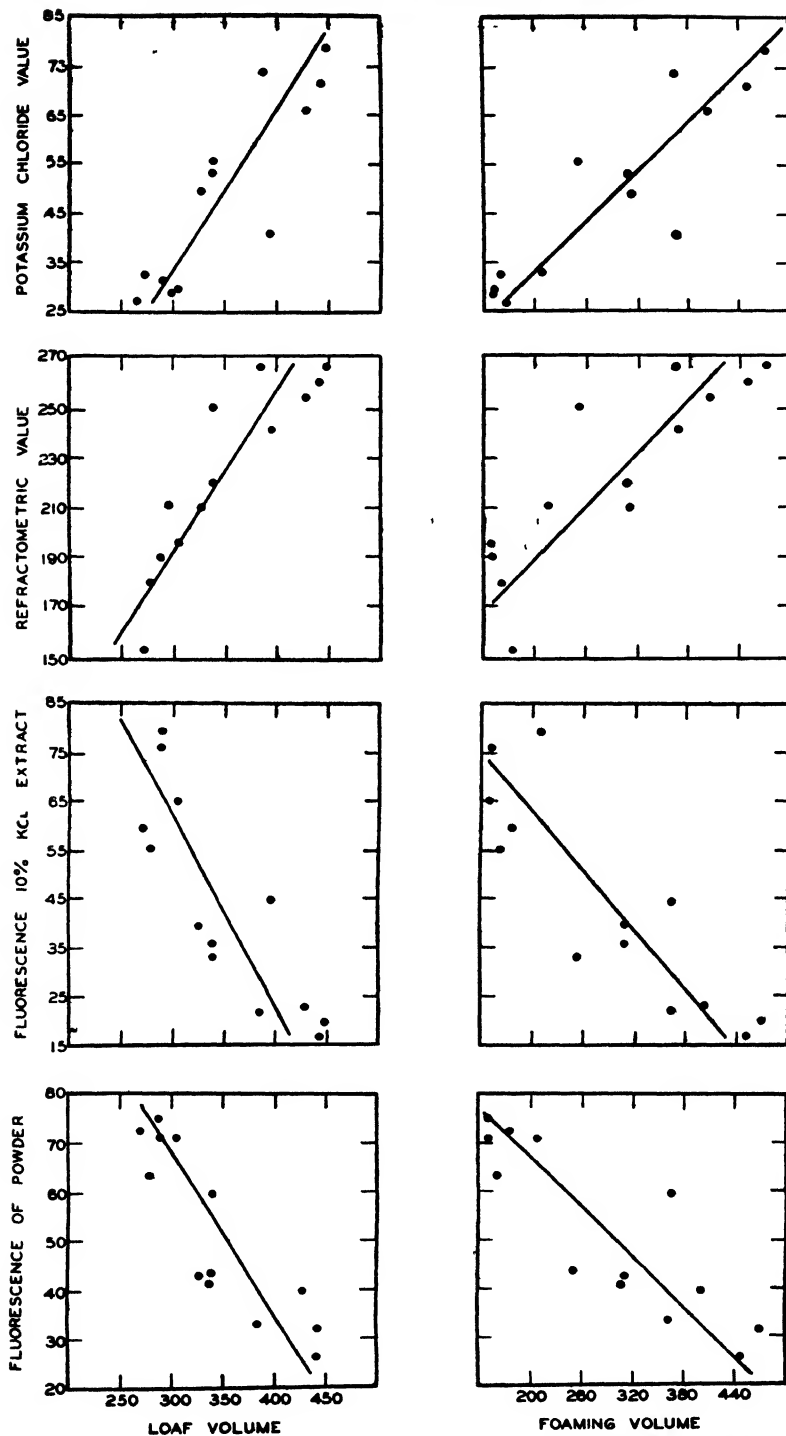
FIG. 1. *Relation of objective tests to foaming and loaf volume measurements.*
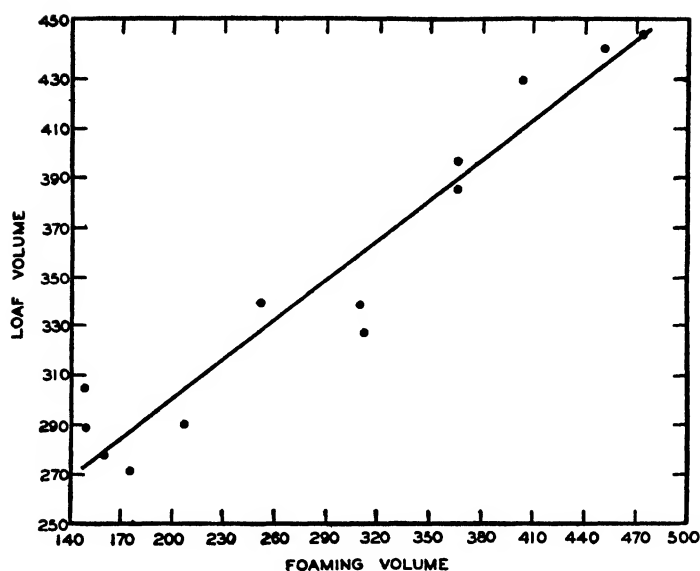
FIG. 2. *Relation between foaming volume and loaf volume measurements of dried egg.*

attributable to the large experimental error in determining loaf volume (standard deviation for replicate loaves 30 ml.; 10% of the mean). Foaming volume was measured with much greater accuracy (standard deviation, 6 ml.; 2% of the mean). In addition to these considerations, foaming volume was more practical for laboratory use; less time and less complicated apparatus were required for making a determination. Therefore it was concluded that foaming volume was the most satisfactory of the methods used in this study for evaluating the baking quality of dried whole egg powder.

Acknowledgments

References

1. BENNION, E. B., HAWTHORNE, J. R., and BATE-SMITH, E. C.   J. Soc. Chem. Ind. 61 : 31-34. 1942.
2. FRYD, C. F. M. and HANSON, S. W. F.   J. Soc. Chem. Ind. 63 : 3-6.   1944.
3. MALLOCH, J. G. and COOK, W. H.   Cereal Chem. 7 : 307-310.   1930.
4. PEARCE, J. A. and THISTLE, M. W.   Can. J. Research, D, 20 : 276-282. 1942.
5. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E.   Can. J. Research, D, 21 : 1-7.   1943.
6. WHITE, W. H. and GRANT, G. A.   Can. J. Research, D, 21 : 203-210. 1943.
7. WOODCOCK, A. H. and REID, M.   Can. J. Research, D, 21 : 389-393.   1943.
8. WOODCOCK, A. H. and TESSIER, H.   Can. J. Research, A, 21 : 75-78.   1943.

PREPARATION OF IRISH MOSS EXTRACTS FOR USE AS A JELLING AND STABILIZING AGENT IN FOODS[1]

By C. O. Fulton[2] and Miss B. Metcalfe[3]

Abstract

Bleached Irish moss suspended on 40-mesh screens was extracted three times with water at 212° F. (100° C.). The second and third extracts were used for extracting fresh batches and by this method solutions containing 1.5 to 1.8% solids were obtained. One percent of activated charcoal mixed with the solution by agitation with air for one-half hour adsorbed all detectable flavours and odours and most of the pigment. The charcoal and suspended plant particles were removed simultaneously by filtration at 50 to 60 lb. pressure with diatomaceous earth of relatively large particle size. Potassium chloride (0.5 gm. per 100 ml.) was added to the hot filtrate, which was then poured into galvanized iron trays, allowed to gel, and frozen in air at 10° F. (−12° C.). The ice was separated mechanically from the contracted sheet of jelly, which lost on the average 90% of the water. At room temperature the rubber-like contracted sheet of jelly was dried, by means of a fan, to a residual moisture content of 8 to 10% in about two hours. The dried sheet was coarsely ground in a Wiley mill.

The resulting product employed in jellied canned chicken was preferred to an agar-agar pack by a consumer's taste panel. In grape jelly it was not a complete substitute for pectin but was considered acceptable as a fruit jelly. In three standard desserts it was not as desirable as gelatine but was considered acceptable as a jellied dessert. The material was effective in stabilizing chocolate milk in the same concentration as a commercial product now on the market.

Introduction

For many years the hot water extract (gelose) of the seaweed, Irish moss (*Chondrus crispus*), has had numerous uses. In pharmaceutical preparations it has been used as an emulsifying agent. It has been employed as a fining agent in brewing, a sizing agent in the textile industry, and a thickening agent for cold water paints.

In the food industry its chief use in recent years has been as a stabilizer for chocolate milk drinks. For this purpose 0.045% is, on the average, sufficient for satisfactory stabilization, and flavours imparted by the gelose are not detectable in the final product. The use of the extract in jellied food products was first suggested in a patent by Leon (4) who indicated that concentrations of from 1 to 2% and the presence of a potassium salt were necessary for satisfactory gels. He also stressed that for jellied food products the extract must be treated with charcoal before drying.

The claims of Leon were confirmed by Reedman and Buckby (7) who successfully used dried charcoal-adsorbed extracts as a substitute for agar-agar in canned chicken and showed that up to 0.5% the addition of potassium

---

chloride to a solution of gelose increased the gel strength in a straight line relationship. However, to obtain satisfactory gels they relied solely on the natural potassium salts present in chicken broth. Their laboratory process produced a very pure extract, but, owing to the cost of drying extracts containing 0.33 to 0.46% solids, the procedure as such did not show evidence of being commercially feasible.

The object of the investigation described herein was to evolve better methods of extraction and filtration; to explore drying techniques other than spray, drum, and tunnel drying; to assess the usefulness of the product as a substitute for agar-agar, pectin, and gelatine; and to determine whether a product with high jelling properties could also be used as a stabilizer for chocolate milk drinks. The value of the material as a substitute for agar-agar was deemed most important in view of the wartime scarcity, but the production of a product of general value as a jelling and stabilizing agent was the over-all objective.

Experimental Procedure and Results

PRODUCTION OF DRIED EXTRACTS

Preparation of Sample

Dried extracts from bleached moss are lighter in colour than those from unbleached moss, and therefore more desirable for commercial purposes. Thus the data contained herein apply chiefly to sun-bleached moss from the province of Prince Edward Island. The unrefined moss as received contains sea-water salts and extraneous matter most of which can be washed off with cold water. Since Haas and Hill (3) have indicated that Irish moss contained both cold-water– and hot-water–soluble polysaccharides, experiments were made to compare the gel strengths of the two fractions.

The apparatus for determining gel strength has been described elsewhere (6). Duplicate determinations were made on quadruplicate samples of 100 ml. of gel that had been allowed to set in sealed 7 oz. lacquered cans for 18 hr. at 45° F. (7° C.).

Preliminary tests showed that a 2% solution of the alcohol precipitate of the solids extracted at 70° F. (21° C.) (the cold fraction) had approximately one-sixth the gel strength of a 2% solution of the alcohol precipitate obtained by extracting the remaining solids in water at 212° F. (100° C.) (the hot fraction). Also since the solids extracted at 70° F. (21° C.) represent a small fraction of the total extractable solids, the total fraction produces gels sufficiently strong for practical purposes. This can be seen in Fig. 1 in which the unautoclaved gel from the hot fraction has a gel strength of 118 units, whereas the gel from the total fraction has a gel strength of 105 units. After the heat treatment the samples in 7 oz. air-exhausted sealed cans were cooled immediately by the introduction of cold water into the retort. After removal from the retort the gels were handled as described above before the gel strength determinations were made.

Further, it was found that the fraction extracted at 70° F. (21° C.) had the property of stabilizing chocolate milk; and thus to obtain an extract that was satisfactory for producing gels and had maximum stabilizing ability, the total fraction, i.e. the hot and cold extract combined, was the material studied in
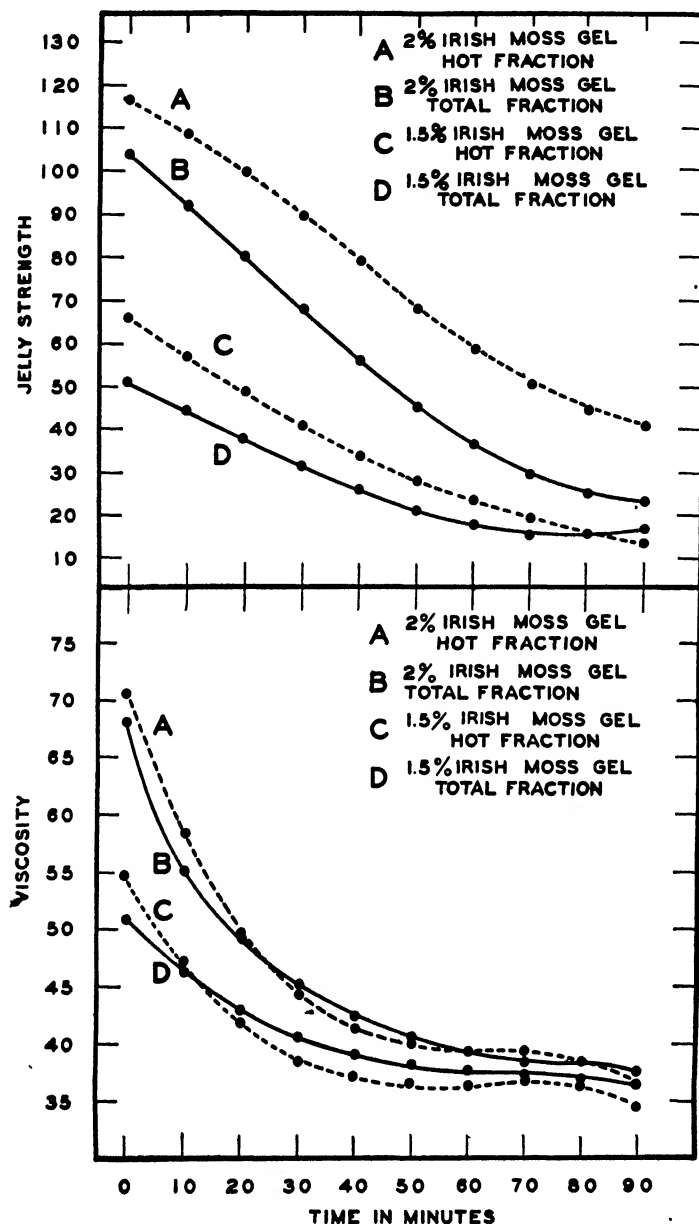

FIG. 1.   *The effect of a temperature of 250° F. (121° C.) on the gel strength and viscosity of two concentrations of both the total and hot fractions.   The points plotted are the averages of eight measurements.*

this investigation.   It was found that a 10 min. preliminary spraying of the bleached moss with water at a temperature not higher than 45° F. (7° C.), washed off sand and sea water salts together with the brownish-yellow oxidized surface of bleached moss and resulted in purer, more colourless extracts; so that strictly speaking the "total fraction" alluded to above refers to the hot-water–soluble material obtainable after the moss has been thoroughly sprayed for 10 min. with water at a temperature not higher than 45° F. (7° C.).

Extraction

Extensive preliminary tests of the effects of time and temperature on the properties of gelose indicated that' the conditions for extraction should be set at not longer than two and one-half hours at a temperature of 212° F. (100° C.).

Reedman and Buckby (7) obtained solutions with 0.33 to 0.47% solids. The amounts extracted were small because of filtration difficulties.   These were assumed to be due to the high viscosity of the solution but in the present investigation it was found that they were caused chiefly by the concentration of insoluble suspended particles.   This is illustrated by the following experiment: 6 gm. of moss was extracted in 300 ml. of hot water by vigorous agitation with a stirring motor.   The larger insoluble portions of the extracted plant were removed by forcing the mixture through a 40-mesh screen, and 200 ml. of the resulting suspension containing 1% Johns-Manville No. 545 filter-aid required 95 sec. to filter through 19 sq. in. of canvas at a pressure of 20 lb. and a temperature of 200° F. (93° C.).   The same volume of the filtered solution was refiltered under the same conditions in six seconds.   Therefore, since the viscosity of each solution was theoretically the same, the tremendous difference in filtering time was due to the high concentration of suspended particles.   It was found that if the whole plant were extracted in circulating hot water and not broken up by the agitation of a stirring motor, most of the soluble solids could be extracted and the concentration of suspended particles minimized so that filtration could be readily accomplished.

Accordingly, a cubical steam-jacketed extractor was constructed with a capacity of 53 kgm. of hot water at 212° F. (100° C.).   Eight 40-mesh screens, 13.5 in. square, were soldered to ½ in. angle-irons to form trays.   These slide into the extractor on ledges at 2 in. intervals.   When the gasket-covered removable end of the extractor is bolted on, the trays are forced against a gasket at the other end, to prevent liquid flowing around the ends of the trays instead of through them.   The half inch contact between the angle-irons and the ledges tends to prevent the liquid flowing around the sides of the trays. The bottom of the extractor slopes from the sides and ends to an outlet at the centre.   The space between the first screen and the bottom enables the liquid in the extractor to be pumped out and circulated back into the top by means of evenly spaced inlets.   On each of the 40-mesh trays are placed 16-mesh screens, separated from the trays by lengths of ½ in. wire soldered to the under surface.   The coarse screens tend to keep the bulk of the moss

off the fine screens, an arrangement that prevents clogging and facilitates draining.

A quantity of dry Irish moss equal to 3% of the weight of the hot water capacity of the extractor was divided into equal portions and placed on eight trays. The moss was thoroughly sprayed with cold water as described above, evenly distributed over the surfaces of the trays, and placed in the extractor. The extractor was then filled with water at 212° F. (100° C.) and the temperature maintained by steam flowing through the jacket. The hot water was circulated through the screen-held moss for one hour. It was then pumped out. This represents the first extraction, and contains from 1.50 to 1.69% solids. However, a considerable volume of liquid fails to drain away from the moss-covered screens, so the extractor was filled from the bottom with water at 190° F. (78° C.) and extracted as before for 15 min. The combined second and third extracts, containing 0.65% solids, were heated to 212° F. (100° C.) and used to extract a fresh batch of moss weighing 2% of the hot water capacity of the extractor. This procedure readily built up a solution containing 1.8% solids after one hour at 212° F. This method gave yields of 40 to 45%.

It can thus be seen that by applying a modified counter-current system of extraction to Irish moss suspended in tanks on 40-mesh screens, readily filterable solutions containing 1.5% solids can be obtained. If screens were not used it would be impossible to circulate water through the impenetrable mass of pulp-like moss, and, as already pointed out, agitation results in filtration difficulties.

Adsorption

The extract obtained as described above has an unpleasant odour and taste, and is unsuitable for making edible gels. Taste panel tests with three dried extracts now on the market showed them unsuitable for use in jellied chicken. These products are not treated with charcoal.

In the present investigation five different charcoals were tested and Darco *S*-51 was selected on the basis of effectiveness, economy, and availability. It was found, that this charcoal could be removed by adding 1.5% Johns-Manville No. 545 filter-aid and filtering through canvas cloths evenly pre-coated with a ⅛ in. layer of Johns-Manville Hyflo filter-aid. This *cannot be accomplished* if, during adsorption, the solution is subjected to vigorous agitation, if it is passed through a centrifugal pump, or otherwise subjected to violent motion.

Accordingly, the hot solution from the extractor containing 1.6 to 1.8% solids was placed in a steam-jacketed vessel, 1 gm. of Darco *S*-51 per 100 ml. of solution was added and mixed for one-half hour by bubbling air into the base of the vessel. The viscous stabilizing nature of the solution tends to prevent charcoal from settling and the agitation from the air is sufficient to produce homogeneity and yet not unduly reduce the particle size of the carbon.

Filtration

The filter press used was a four-plate, steam-jacketed model of standard design with eight filtering surfaces 4⅞ by 4⅞ in. These were given the filter-aid precoat mentioned above; this was prevented from dropping off the cloths by blowing air at 20 lb. pressure through the press until the solution to be filtered was switched into the line at a higher pressure. If the precoat fails to cover the cloths completely the first portion of the filtered solution will be grey in colour owing to small charcoal particles, and subsequent filtering through a precoat of Hyflo filter-aid *fails* to remove them.

Two grams of Johns-Manville No. 545 filter-aid per 100 ml. was added to the liquid after charcoal adsorption, and thoroughly mixed by agitation with air. The mixture was allowed to flow by gravity into the blow case, and the charcoal and insoluble plant particles were filtered out simultaneously at a pressure of 50 to 60 lb. The filtered solution is tasteless and odourless but is slightly yellow in colour when viewed at depths greater than 3 in. When dried and ground the material has a slightly grey appearance indicating that probably very small amounts of charcoal remain in the solution after filtration.

Drying

The procedure finally adopted was as follows: to the hot charcoal-adsorbed, filtered liquid containing 1 6 to 1 8% solids (previously determined) was added a hot solution containing one part potassium chloride for every three parts of solids in the extract. The hot solution was stirred vigorously for five minutes to ensure complete mixing. After this addition the total solids concentration must not be lower than 2%. It is necessary to add hot potassium chloride solution to hot gelose to prevent the formation of lumps of jelly that require much heat and agitation to dissolve. With some extracts a slight precipitate formed after the addition of potassium chloride, especially if the solution remained hot for periods of longer than one-half hour. For the production of edible gels this precipitate has no practical significance, but if the material is to be used for bacteriological media the precipitate must be removed by filtration.

The extract was then poured into galvanized iron trays 33 by 20 in. to a depth of ⅜ in. in a room at 45° F. (7° C.) and as free from dust as possible. The trays of clear, colourless, extremely firm jelly were then placed on wooden racks at 10° F. (−12° C.) and left in the freezing room until the temperature of the jelly was just below 32° F. (0° C.). Then a handful of crushed ice was scattered over the surface to prevent supercooling. When freezing is complete a concentrated layer of potassium chloride and gelose is sandwiched between two layers of almost pure ice. The ice can be cracked and removed leaving the rubber-like concentrated gelose–potassium-chloride sheet in one piece. Table I gives the percentage of water lost by freezing at different temperatures and depths of gel and shows that at 10° F. (−12° C.) and a depth of ⅜ in. the highest percentage of water was lost. Table II shows that the resulting product contains about 80% moisture. At room temp-

TABLE I

EFFECT OF TEMPERATURE AND DEPTH OF GEL ON WATER LOST IN FREEZING

Temperature, °F.	Depth of gel, in.	Weight of jelly, oz.	Weight of wet gelose–potassium-chloride layer after ice removed, oz.	Water lost during freezing process, %
20	1/4	88	8	91
	3/8	129	12	91
	1/2	177	18	90
10	1/4	82	6	93
	3/8	130	8	94
	1/2	154	10	94
0	1/4	90	12	87
	3/8	134	34	75
	1/2	179	75	58

TABLE II

RESULTS IN DUPLICATE OF DRYING HOT SOLUTIONS CONTAINING 1.5% GELOSE AND 0 5% POTASSIUM CHLORIDE BY TWO DIFFERENT FREEZING TECHNIQUES AT 20° F. (−7° C.)

Items considered	Ice removed mechanically in the cold		Ice removed by melting in air at 70° F. (21° C.) (Japanese agar method)	
Wt. of hot solution, gm.	400	400	400	400
Wt. of jelly after setting at room temp., gm.	383	386	388	387
Wt. of tray contents after freezing, gm.	367	368	369	369
Wt. of water lost by freezing, gm.	328	328	232	218
Water lost during freezing process, %	90.8	90.3	66.5	65.0
Wt. of resulting product, gm.	37	39	134	140
Moisture content of resulting product, %	78.3	79.4	94.0	94.3
Drying time (fan, at room temp.), hr.	2¾	2¾	4¾	4¾
Wt. of dried product, gm.	8 8	8.9	7.6	7.7
Final moisture content, %	8	8	8	8
Jelly strength of 2% solution after reconstituting, gm. of mercury		142		61

erature the sheet was dried, by means of a fan, to 8 to 10% moisture in about two hours.

In the freezing technique described above there are two pitfalls. One is supercooling and the other the seemingly random formation of ice crystals within the concentrated sheet of jelly. If the surface of the gel is not seeded with ice crystals it becomes supercooled and when freezing occurs, micro-crystals are formed throughout the whole gel structure and mechanical separation is impossible. Continuous agitation of the trays during cooling and freezing to prevent supercooling was not as successful nor as simple as seeding with small pieces of ice.

The presence of many entrapped ice crystals causes drying difficulties: mechanical separation of the ice and jelly layers is hindered by protruding crystals, drying time is increased, and some potassium chloride and cold-water–soluble gelose is lost when the crystals melt.

The greatest single factor causing this undesirable condition is a low jelly strength that may be due either to low concentrations of gelose or potassium chloride, or to gelose that has been damaged by too much heat. Fig. 1 shows the effect of heat and time on gel strength and viscosity: gel strengths of 100 units and up were most suitable; gels weaker than 100 units had progressively more entrapped crystals; and at a gel strength less than 55 units neither the hot fraction nor the total fraction could be satisfactorily dried by the freezing technique.

Entrapped crystals were also caused by the following treatments:

Treatments	Effects
Pin-pricks in jelly surface	Crystals
Floor dust on tray before pouring	Many crystals
sprinkled on solution before setting	Many crystals
sprinkled on jelly after setting	No effect
Iron filings mixed with hot gel	Many crystals
Sawdust mixed with hot gel	Many crystals
Control	Two crystals

In the freezing technique described the potassium-chloride–gelose mixture contained 0.5% potassium chloride, because in preliminary taste panel tests it was found that above this level potassium chloride is unpleasantly salty and to some palates slightly bitter.

The goal of a 'perfect freeze', i.e. one in which no ice crystals were entrapped, occurred with two out of 20 batches even when no precautions were taken to prevent dust falling on the freshly poured trays, and depths up to ½ in. were frozen at 0° F. (−18° C.), 10° F. (−12° C.), and 20° F. (−7° C.). These perfect batches contained 1.5% gelose and 0.5% potassium chloride and did not have an unusual jelly strength. It would seem therefore that there are unknown factors inherent in the gelose that cause variations in the number of crystals entrapped while freezing. However, the results given in Table I are representative of what is achieved on the average by the freezing technique described.

Table II compares the method of freezing and drying described above to the method used in Japan for Japanese agar. It can be seen that mechanical separation of the ice rather than the melting of the ice is essential with Irish

moss in order to prevent loss of potassium chloride and the cold-water–soluble fraction. Another advantage is that the jelly does not imbibe melted ice, which must eventually be evaporated.

After drying, the sheets were coarsely ground in a Wiley mill, for it was observed that finely ground material had a tendency to form lumps when mixed with water.

Properties of the Dried Product

This product dissolves in water at 160° F. (71° C.) without lumping and in this respect is superior to the commercial preparations now available.

A 2% gel melts between 130° F. (54° C.) and 140° F. (60° C.) and sets between 100° F. (38° C.) and 110° F. (43° C.). As shown in Fig. 1 the gel strength is slowly reduced by high temperature whereas the viscosity as measured in seconds through a 100 ml. Dudley pipette at 131° F. (55° C.) is rapidly reduced.

Perhaps the most drastic heat treatment to which the product might be subjected would be in the preparation of 6-lb. tins of jellied canned pork, which are sterilized at 230° F. (110° C.) for 200 min. Pork broth was not available; chicken broth at a lower pH was substituted, and the effect of the time–temperature conditions of the canned pork process was determined on the Irish moss preparation. Fig. 2 shows the loss in gel strength of two concentrations of commercial shredded agar and two concentrations of Irish moss extract. Under these conditions agar is more stable, yet at the end of the treatment the Irish moss gel was sufficiently strong at the 2% level to maintain its structure in air at 120° F.
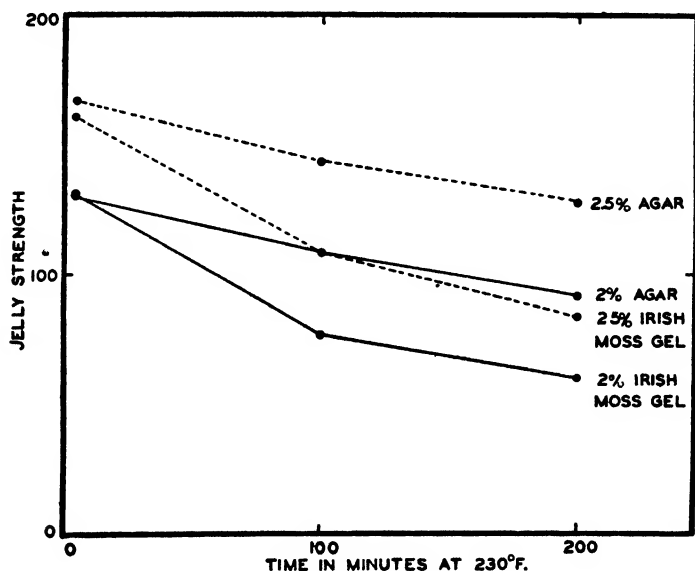

FIG. 2. *The effect of prolonged heat treatment (230° F. or 110° C.) on the gel strength of two concentrations of both agar-agar and the total fraction of the Irish moss preparation.*

Uses

As a Substitute for Agar-agar in Jellied Meats

Grade A milk-fed chickens were prepared by standard methods (1). After cooking, the meat was removed from the birds, pooled, and $3\frac{1}{2}$ oz. of meat, half light and half dark, packed into 7 oz. cans. One per cent of sodium chloride was added to the broth, which was then divided into two portions. To one was added 2 gm. per 100 ml. Irish moss preparation (approximately 69% gelose, 23% potassium chloride, and 8% moisture) and to the other 2 gm. per 100 ml. commercial shredded Japanese agar. Three and one-half ounce portions of each of these broths were added to cans containing meat. The cans were placed in flowing steam for 15 min., closed, and retorted 45 min. at 250° F. (121° C.).

A can of each product was given, together with a questionnaire, to 20 families, and 45 persons sampled the canned meats in their homes at meal time. The Irish moss preparation was preferred by 71%, 18% preferred agar, and 11% could find no difference. Since the gel strength of the moss preparation after retorting was weaker than that of agar, it was thought that perhaps the preference was given to Irish moss because it was softer and more reminiscent of gelatine. Accordingly, a similar pack was prepared except that the broth had $1\frac{1}{2}$ gm. of agar per 100 ml. This was compared to the Irish moss preparation by a group of 13 Dominion Government poultry inspectors: 64% preferred the moss preparation and 36% the agar preparation.

As a Substitute for Bacteriological Agar

Walker and Day (8) tested commercial 'Carragar' for its bacteriological potentialities. Since this product contained only a small amount of potassium chloride, gels as strong as agar were not obtained and the fortification of the product with a small quantity of agar was recommended.

The physical properties of the product described herein indicated two slight disadvantages for bacteriological work. One is the fact that the melting point of even a 2% solution that had been autoclaved 20 min. was between 122° F. (50° C.) and 140° F. (60° C.). This indicates that the product could not be used for the isolation of thermophiles. This disadvantage is partially balanced by the fact that unusual solid media, containing heat coagulable substances such as serum, could be remelted in a water-bath at 131° F. (55° C.) and used again for the isolation of mesophylic and psychrophylic organisms, whereas the high melting point of agar makes such a procedure impossible.

Another slight disadvantage is indicated in Fig. 1. The viscosity of a $1\frac{1}{2}$% solution after 20 min. autoclaving is 42 sec. at 131° F. (55° C.) whereas that of $1\frac{1}{2}$% agar under similar conditions is 35 sec., and of water 34 sec. Thus the 'poured plate' technique would require more thorough mixing than is necessary with media containing agar, but this disadvantage is not considered serious.

A solution containing $1\frac{1}{2}$% Irish moss preparation, which under normal atmospheric conditions contains 8% moisture, would be composed of 1.035%

gelose and 0.345% potassium chloride. It is our opinion that this concentration of potassium chloride would not have a significant effect on the morphological or physiological characteristics of micro-organisms.

As a Substitute for Gelatine

Four gelatine desserts were prepared from standard recipes. The same desserts were prepared using the Irish moss preparation. Gelatine was preferred by 67% of 16 tasters, but all stated that the other samples were acceptable as jellied desserts. To gel 100 ml. of liquid with gelatine required 1.5 gm. To give a gel of the same volume with approximately the same strength required 0.6 gm. of the moss preparation. Tasters preferred the texture of the gelatine desserts, which seemed less brittle than those of Irish moss. On the basis of flavour and appearance there was no significant difference between the two. When the moss preparation was beaten with egg-white, and allowed to stand in the cold over-night, undesirable syneresis occurred.

As a Substitute for Pectin

Since sugar and acid are not necessary to make jelly from Irish moss, it was thought that a superior jelly with more natural fruit flavour could be prepared. Taste panel results contradicted this supposition, for the flavour produced by the combination of the high sugar and acid content of pectin jellies was preferred by 94% of 16 tasters. When 50% by weight of sucrose was added to grape juice with the moss preparation the jelly was too sweet. If the equivalent weight of citric acid found in pectin jellies was added, and the jelly boiled one minute for sterilization purposes, the moss preparation failed to gel on cooling.

In grape juice the nearest approach to the flavour of a pectin jelly was obtained by adding 30% sugar and relying on the natural acids in the juice to produce tartness. Even under these conditions 75% of 16 tasters preferred pectin jelly. However, 81% classed jellies made from Irish moss as acceptable. To gel 100 ml. of juice containing 50% by weight of sugar required 7.8 gm. of pectin crystals. To gel 100 ml. of juice containing 30% sugar by weight required 1 gm. of moss preparation or approximately one-eighth as much.

As a Stabilizer for Chocolate Milk Drinks

A comparison between the stabilizing properties of the present preparation and a dried commercial extract by a procedure described in a personal communication kindly sent by the Kraft Cheese Company, showed no difference between the two products, 0.225 gm. per 500 gm. of milk being required for complete stabilization, in both instances. Since the commercial product did not contain added potassium chloride, and had 6% moisture as compared to 8%, it would seem that the actual gelose in our preparation was superior as a stabilizer. The reason for this may be that the high temperatures used to dry the commercial extracts are detrimental, but time did not permit this point to be investigated.

Other Uses

Attempts were made to ascertain if edible solutions such as extracts of tea, coffee, or ground beef, and slurries such as finely ground beef, carrots, yeast cells, tomato juice, and orange juice could be dried by the freezing technique.

Accordingly 2 gm. of moss preparation per 100 ml. of solution was added at room temperature, thoroughly mixed, and permitted to stand for 20 min. The standing period of 20 min. caused the granules of the Irish moss preparation to swell and resulted in a shorter heating period to effect solution, a few minutes at 160° F. (71° C.) being sufficient. The solution was then poured into trays, allowed to set in the usual manner, frozen at 10° F. (−12° C.), and the concentrated sheet of solids removed and dried. All of the above-mentioned materials could be successfully dried by the freezing method except tomato juice and orange juice, which failed to separate on freezing possibly because the natural acids weakened the gel structure during the heating period.

Discussion

Of the various steps in the procedure for obtaining the Irish moss preparation perhaps the freezing technique is deserving of further elaboration. Within wide limits the behaviour of gels from Irish moss extracts on freezing corresponds to the observations on gelatine made by Moran (5) whose results correspond within narrow limits to those of Gryuner and Gorshkov (2) for the freezing of agar-agar jellies extracted from *Anfeltia plicata* [*sic*].

Moran concluded that when gelatine gels are frozen there are two different sites of crystallization centres; those that are inside the jelly and those that are outside. The site of crystallization depended on the jelly strength and on the speed of freezing. In jellies with a gelatine content lower than 12% the internal centres of crystallization become more active. When 12% jellies were rapidly frozen by immersion in liquid air no ice formed on the surface. At temperatures from 12.2° F. (−11° C.) and up, progressively more ice formed on the surface than on the inside, until at 27.6° F. (−2° C.) a thick layer of ice formed on the surface with no ice inside.

Gels from Irish moss apparently differ from those of agar and gelatine in that, on freezing, centres of crystallization can be induced to form only on the surfaces at far lower temperatures, 0° F. (−18° C.) having been occasionally recorded as compared to 27.6° F. (−2° C.) for Moran's gelatine and 23° F. (−5° C.) for the agar used by Gryuner and Gorshkov. Since neither Moran nor the Russian workers mention taking steps to prevent supercooling, it was thought that the present method of freezing applied to agar would prevent the formation of internal centres of crystallization at lower temperatures. This, however, was not the case, although small amounts of surface ice did appear on agar gels at concentrations of 1, 1½, and 2% frozen in air at 20° F. (−7° C.). Zhelezhov (9) states that the freezing out of agar gels depends on the salt concentration, yet in our experience the addition of sodium or potassium chloride to agar gels and the application of ice crystals to prevent super-

cooling did not result in 'freezes' from which agar and ice could be readily separated without melting away the ice. Therefore it would seem that the difference between the conditions necessary for ideal freezing of Irish moss gels and those for gelatine and agar gels is due to inherent and unique properties of the polysaccharide.

If full advantage were taken of Canadian winter conditions to supplement refrigeration it is thought possible that a satisfactory jelling and chocolate milk stabilizing product could be manufactured on the commercial scale by the freezing method.

Acknowledgments

Grateful acknowledgment is made to Dr. A. W. H. Needler, Director, Atlantic Biological Station, through whose aid samples of moss were obtained. We are indebted to Dr. W. H. Cook, Director of Division of Applied Biology, National Research Council, who suggested drying gels by freezing. Thanks are extended to Mr. W. Mouré, for assistance in the early stages of the work. The authors are also indebted to the Kraft Cheese Company, the Krimko Company, and the MacLean's Cocoa Mills for supplying samples of their commercial products.

References

1. CAN. DEPT. AGR. Dominion Government Regulations for the Packing, Grading and Marketing of Canned Poultry. May, 1943.
2. GRYUNER, V. and GORSHKOV, I. J. Applied Chem. (U.S.S.R.), 10 : 2054-2063. 1937.
3. HAAS, P. and HILL, T. G. Ann. Applied Biol. 7 : 352-362. 1921.
4. LEON, J. H. Fr. Pat. 680,188. Aug. 12, 1929.
5. MORAN, T. Proc. Roy. Soc. (London), A, 112 : 30-46. 1926.
6. REEDMAN, E. J. Can. J. Research, D, 21 : 324-331. 1943.
7. REEDMAN, E. J. and BUCKBY, L. Can. J. Research, D, 21 : 348-357. 1943.
8. WALKER, A. W. and DAY, A. A. Food Research, 8 : 435-443. 1943.
9. ZHELEZKOV, P. S. Colloid J. (U.S.S.R.), 6 : 403-407. 1940.

RATION BISCUITS

III. EFFECT OF MOISTURE CONTENT ON KEEPING QUALITY

By J. B. Marshall, G. A. Grant, and W. Harold White

RATION BISCUITS

III. EFFECT OF MOISTURE CONTENT ON KEEPING QUALITY[1]

By J. B. Marshall[2], G. A. Grant[3], and W. Harold White[4]

Abstract

Ground biscuits made with two types of shortening were adjusted to moisture contents of approximately 0, 3, 6, 9, 12, 15, and 18% and stored at 43.3° C. (110° F.) for 56 wk. Keeping quality was assessed by flavour tests, peroxide oxygen, pH, and fluorescence measurements. Although the behaviour of the biscuits was similar, and variations in moisture content had small effect on flavour scores, Biscuit A, made with the more stable shortening, gave differentially higher scores at moisture levels below 6%. The evidence from the objective measurements indicated also that a moisture content of about 6% was most suitable for storage. Changes in the fat component as measured by peroxide oxygen were inhibited as the moisture content was increased over the range studied. The formation of fluorescing substances reached a maximum at a moisture content of 12% and decreased somewhat at higher levels.

Introduction

The preservation of foodstuffs by dehydration depends primarily on the reduction and control of moisture content (5, 7). In the first paper of this series it was shown that the storage life of ration biscuits containing protein-rich supplements could be extended by maintaining the moisture content below 6%. It was assumed that deterioration of the protein and carbohydrates preceded that of the fat fraction (4). However, it has been reported that more rapid deterioration of the fat occurred in milk (3) and in grain products (1) when the moisture content was reduced, and in ground cracker material stored over concentrated sulphuric acid (6). Thus for material of mixed composition, such as ration biscuits, the most satisfactory moisture content for storage should be one that achieves a balance between the rates of deterioration of the fat and non-fat components. The present study was undertaken to ascertain this optimum moisture content.

Materials and Procedure

The biscuits were prepared by a commercial manufacturer according to a simple formula consisting of 50 lb. of soft wheat flour, 5 lb. of shortening, and 6 oz. of soda. Two types of shortening were used, (A) a highly stable hydrogenated vegetable oil product and (B) a much less stable, compounded animal-vegetable shortening, the biscuits made with these shortenings being designated Biscuit A and Biscuit B respectively. Samples of the biscuits were

[2]   *Biologist, Food Investigations.*

[3]   *Laboratory Steward, Food Investigations.*

[4]   *Formerly Biochemist, Food Investigations, now Director of Research, F. W. Horner, Limited, Montreal.*

ground mechanically and adjusted to moisture levels of approximately 0, 3, 6, 9, 12, 15, and 18% by the method described for dried whole egg powder (7).　Calcium chloride was replaced by phosphorous pentoxide to obtain the two lowest levels.　The actual values are given in Table I.　Samples of about

TABLE I

MOISTURE CONTENTS OF BISCUITS

Biscuit	Moisture contents, %						
A	0.2	3.1	5.9	9.0	12.0	14.9	17.8
B	0.2	3.0	6.3	9.0	12.0	15.0	18.0

100 gm. of the adjusted material were placed in glassine bags to prevent direct contact with the tin-plate containers (No. 1 size cans) in which they were hermetically sealed, and stored at 43.3° C. (110° F.)　Material for initial examination and 10 sampling times were provided for each biscuit at each of the above moisture contents.

Changes in the palatability of the material during storage were assessed by a panel of 16 tasters who scored the samples according to the following scale: 10, excellent, fresh flavour and odour; 8, good, no off-flavour or odour; 6, fair, slight off-flavour and odour; 4, poor, marked off-flavour and odour; 2, very poor, offensive flavour and odour; 0, inedible.

Objective measurements of deterioration were made by determining the peroxide oxygen values of the extracted fat and the development of fluorescing substances by methods used in previous studies of this series (3, 5).　The pH of the sodium chloride extracts used in making the fluorescence measurements was also measured.

Results

The results of flavour score, fluorescence, and pH measurements are summarized in Table II to indicate the general effect of moisture content and storage time on the biscuits.　The biscuit means are averages over all sample times and moisture levels; those for moisture content and storage time are the averages of the marginal totals of data summary Tables V, VI, and VII, given in the Appendix.　This method of presentation provides a convenient condensation of the data for the entire experiment in a manner that shows the effects of the experimental variables.　The significance of these was assessed statistically by means of analysis of variance shown in Table III.

The peroxide oxygen results given in Table IV were omitted in the summary Tables II and III as the data are not amenable to analysis of variance.

Flavour

Biscuit *A* made with the more stable shortening had a higher mean flavour score than Biscuit *B* made with the compound animal–vegetable shortening.

TABLE II

MEAN VALUES OF FLAVOUR SCORES, FLUORESCENCE, AND pH MEASUREMENTS OF BISCUITS
STORED AT 43.3° C. (110° F.)

Variable	Flavour		Fluorescence		pH	
	Biscuit A	Biscuit B	Biscuit A	Biscuit B	Biscuit A	Biscuit B
Material*	6.6	6.1	39.0	36.0	7.37	7.37
Moisture, %**						
0	7.1	5.9	35.5	32.0	7.06	7.48
3	7.1	6.0	35.9	33.2	7.46	7.48
6	6.7	6.2	37.8	34.2	7.46	7.40
9	6.7	6.2	42.1	39.3	7.44	7.36
12	6.5	6.2	44.5	41.5	7.37	7.34
15	6.4	6.2	39.7	37.5	7.44	7.32
18	5.9	5.7	37.2	34.6	7.30	7.28
Time, wk.***						
Initial	8.0	7.9	34.4	30.0	7.88	8.05
2	8.0	7.2	31.1	28.2	7.51	7.61
4	7.4	6.5	41.0	36.4	7.39	7.40
6	7.2	6.6	38.3	33.5	7.24	7.21
8	7.4	6.4	40.3	36.5	7.48	7.49
12	7.2	6.3	40.0	35.6	7.54	7.54
18	6.2	5.5	34.2	30.6	7.06	7.28
24	6.4	5.9	40.0	36.9	7 44	7.46
32	6.3	6.2	44.0	43.6	6.98	6.73
44	3.3	2.9	42.4	43.4	7.02	6.92
52	5.6	5.5	43.5	41.4	7.44	7.45

   * *Data averaged for all moisture levels and sampling times.*
   ** *Data averaged for all sampling times.*
   *** *Data averaged for seven moisture levels.*

TABLE III

ANALYSES OF VARIANCE OF FLAVOUR SCORES, FLUORESCENCE VALUES, AND pH DATA FOR
BISCUITS, ADJUSTED TO VARIOUS MOISTURE CONTENTS AND STORED
AT 43.3° C. (110° F.)

Source of variance	Degrees of freedom	Mean squares		
		Flavour score	Fluorescence	pH
Biscuits	1	11.56*	333.13**	0.08
Sampling times	10	23.21**	300.74**	1.31**
Moisture levels	6		253.66**	0.11
18% *vs.* others	1	8.15*		
Others	5	0.18		
Biscuits × times	10	0.37*	12.05*	0.05
Biscuits × moisture levels	6	1.00**	1.28	0.17
Moisture levels × times	60	0.21**	18.65**	0.06
Biscuits × moisture levels × times	60	0.12	4.96	0.12

   * *Indicates 5% level of significance.*
   ** *Indicates 1% level of significance.*

TABLE IV

PEROXIDE OXYGEN VALUES (ML. $0.002\ N$ THIOSULPHATE PER GM.) OF FAT EXTRACTED FROM BISCUITS ADJUSTED TO VARIOUS MOISTURE CONTENTS AND STORED AT 43.3° C. (110° F.)

Storage time, wk.	Moisture content, %						
	0	3	6	9	12	15	18
Biscuit A							
Initial	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
4	4	2	0	0	0	0	0
6	8	6	0	0	0	0	0
8	12	8	0	0	0	0	0
12	16	12	6	0	0	0	0
18	28	18	10	0	0	0	0
24	25	26	15	3	2	0	2
32	22	29	20	4	3	0	2
44	14	19	14	5	3	2	2
56	12	19	14	4	3	2	2
Biscuit B							
Initial	0	0	0	0	0	0	0
2	10'	8	4	0	0	0	0
4	20	18	10	0	0	0	0
6	56	46	17	0	0	0	0
8	37	28	32	0	0	0	0
12	26	22	26	24	0	0	0
18	14	13	20	6	0	0	0
24	11	11	17	4	5	2	2
32	8	11	16	4	7	0	0
44	12	13	13	7	2	4	4
56	11	12	4	3	4	3	0

This difference was greater at the lower moisture levels (Fig. 1) and during the first 18 wk. of storage (Table II). Analysis of variance (Table III) showed that the two biscuits deteriorated at different rates with time and
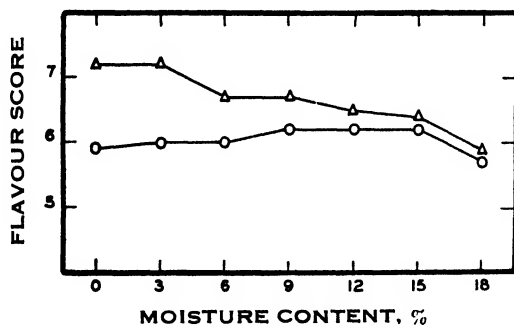
FIG. 1. *The effect of moisture content on the mean flavour score.* △ = *Biscuit A;* ○ = *Biscuit B.*

moisture content. Although mean flavour scores (Table II) decreased with increasing moisture content the only significant difference was between the 18% level and the others. The anomalous flavour scores at 44 and 56 wk. may be accounted for in part by the fact that regular members of the panel were on holiday leave.

Fluorescence

The development of fluorescing substances was somewhat irregular (Table II) but followed a similar trend in both biscuits. Increasing the moisture levels resulted in maximum fluorescence values at 12% with a decline at the higher levels (Fig. 2). Grouping the data according to similarity of the trends
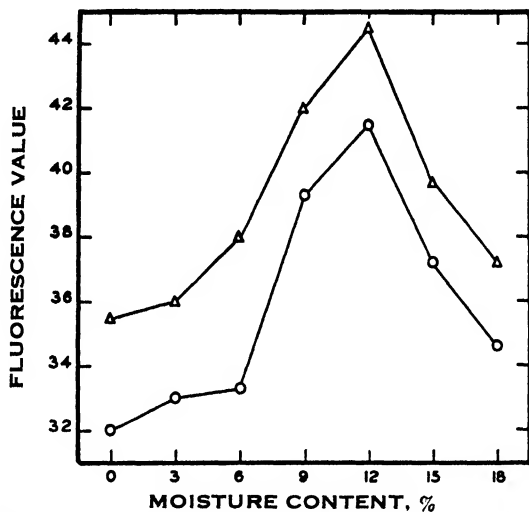
FIG. 2.   *The effect of moisture content on the mean fluorescence values.*   △ = *Biscuit A;* ○ = *Biscuit B.*

for the sampling times showed that initially and during the first four weeks of storage, variations in the moisture content from 0 to 12% had little effect on fluorescence values, while above this level the values progressively decreased (Fig. 3); subsequent samplings attained maximum values at 12% moisture. Analysis of variance established the significance of these trends and the differential behaviour of the Biscuits A and B during storage; the latter resulting from smaller differences between the material at the later sampling times (Table II).

With these biscuits, fluorescence measurements appear to have measured changes that were not detected by the tasters. Biscuit A, having the higher flavour score also had higher fluorescence values.

pH

The mean values for pH of the saline extracts shown in Table II indicate an increase of acidity with storage time, but the data were much too variable to establish significant trends.
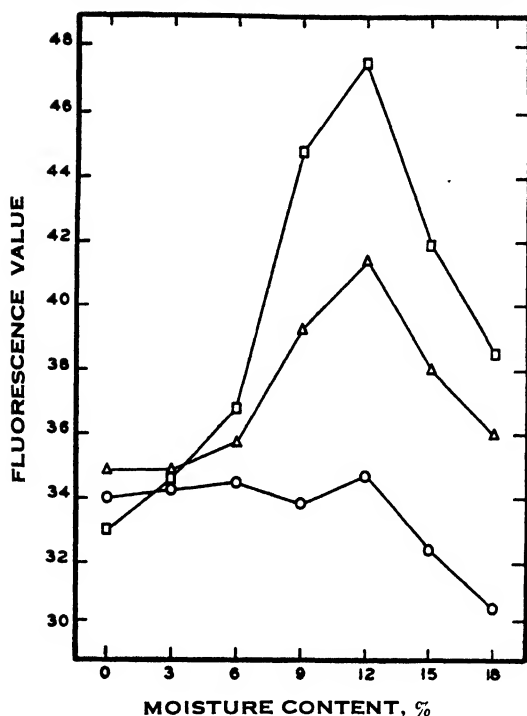
Fig. 3. *The effect of moisture content on the development of fluorescence values.* ○ = *mean for initial, two, and four week samples;* △ = *mean for six and eight week samples;* □ = *mean for 12 to 56 week samples.*

Peroxide Oxygen

Data showing the effect of moisture content on the development of peroxide oxygen in the fats are given in Table IV.   The fats from Biscuit *A* made with the more stable vegetable oil shortening had longer induction periods than the fats made from Biscuit *B* at comparable moisture levels.   The latter also reached much higher values than Biscuit *A* before decomposition of the peroxides commenced.   Moisture content had a marked effect in the region of 6 to 9%; the fats from all samples of the 6% or lower being much less stable than those of the 9% or higher.

Discussion

The effect of moisture content on the deterioration in flavour of the biscuits used in this experiment was not as pronounced as the changes that were detected by objective measurements.   The results have been presented without attempting to correlate objective and organoleptic data as the latter reflect the condition of the entire biscuit at the time of tasting, while peroxide oxygen values and fluorescence measurements assess changes in the fat and non-fat components.   Thus while the development of peroxide oxygen values at moisture contents below 6% was accompanied by a decrease in flavour scores, flavour also deteriorated at the higher moisture levels, although peroxide

oxygen did not develop or accumulate in appreciable amounts. Changes assessed by fluorescence measurements were greater at the higher moisture levels and had reached considerable magnitude before appreciable flavour changes were detected.

The results of this investigation indicate that a moisture content of about 6% was most suitable for the storage of the biscuits.

Acknowledgments

References

1. FINE, M. S. and OLSEN, A. G. Ind. Eng. Chem. 20 : 652-654. 1928.
2. GRANT, G. A., MARSHALL, J. B., and WHITE, W. H. Can. J. Research, F, 23 : 123-131. 1945.
3. HOLM, G. E., WRIGHT, P. A., and GREENBANK, G. R. J. Dairy Sci. 10 : 33-40. 1927.
4. PEARCE, J. A. and MARSHALL, J. B. Can. J. Research, F, 23 : 22-38. 1945.
5. TOMKINS, R. G., MAPSON, L. W., ALLEN, R. J. L., WAGER, H. G., and BARKER, J. J. Soc. Chem. Ind. (Trans.) 63 : 225-231. 1944.
6. TRIEBOLD, H. O. and BAILEY, C. H. Cereal Chem. 9 : 91-106. 1932.
7. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 211-222. 1943.

Appendix

TABLE V

FLAVOUR SCORES OF BISCUITS ADJUSTED TO VARIOUS MOISTURE CONTENTS AND STORED AT 43.3° C. (110° F.)

Storage time, wk.	Moisture content, %							
	0	3	6	9	12	15	18	Mean
Biscuit A								
Initial	7.5	8.2	8.4	8.1	7.9	8.1	7.7	8.0
2	8.3	8.4	8.1	8.1	7.7	7.9	7.2	8.0
4	8.0	8.3	7.9	7.8	1.1	7.0	6.0	7.4
6	7.8	7.9	7.4	7.3	7.3	6.5	6.1	7.2
8	7.9	7.9	7.6	7.1	7.1	7.4	6.9	7.4
12	7.9	7.1	7.1	7.2	7.0	6.3	6.6	7.2
18	6.7	6.7	5.8	6.2	5.9	5.9	5.9	6.2
24	7.4	7.4	6.4	6.2	6.0	6.4	5.1	6.4
32	6.6	6.6	6.3	6.7	6.1	6.3	5.5	6.3
44	3.9	3.9	3.4	3.4	3.5	2.6	2.4	3.3
56	5.9	5.5	5.7	5.9	5.6	5.7	5.1	5.6
Mean	7.1	7.1	6.7	6.7	6.5	6.4	6.9	6.6

TABLE V—*Concluded*

FLAVOUR SCORES OF BISCUITS ADJUSTED TO VARIOUS MOISTURE CONTENTS AND STORED AT 43.3° C. (110° F.)—*Concluded*

Storage time, wk.	Moisture content, %							
	0	3	6	9	12	15	18	Mean
Biscuit B								
Initial	7 1	8 1	8 1	8 2	7 8	8 2	7 6	7 9
2	7 6	7 2	6 7	7 4	6 7	7 3	7 3	7 2
4	6 5	6 6	6 8	6 4	7 1	6 5	5 8	6 5
6	6 2	5 6	6 9	6 8	7 1	6 8	6 5	6 6
8	5 8	6 2	6 1	6 3	7 2	7 1	6 2	6 4
12	5 4	6 1	6 1	6 4	6 6	6 9	6 4	6 3
18	5 4	5 1	5 6	5 8	5 9	5 4	5 3	5 5
24	6 8	6 8	6 4	5 9	4 2	5 9	5 1	5 9
32	5 8	6 3	6 6	6 4	6 6	6 3	5 3	6 2
44	3 1	2 7	3 1	3 1	3 4	2 5	2 3	2 9
56	5 4	5 2	5 6	6 0	5 8	5 8	4 9	5 5
Mean	5 9	6 0	6 2	6 2	6 2	6 2	5 7	6 1

TABLE VI

FLUORESCENCE VALUES OF BISCUITS ADJUSTED TO VARIOUS MOISTURE CONTENTS AND STORED AT 43 3° C (110° F)

Storage time, wk	Moisture content, %							
	0	3	6	9	12	15	18	Mean
Biscuit A								
Initial	34 5	34 5	34 5	34 5	35 5	34 5	32 5	34 4
2	33 5	33 0	33 5	30 5	30 5	29 5	27 0	31 1
4	39 0	41 0	41 0	40 5	45 5	40 5	38 5	41 0
6	37 5	37 0	35 5	40 0	43 5	38 5	36 0	38 3
8	36 0	38 0	39 0	41 5	46 0	41 5	40 5	40 3
12	34 5	37 5	37 5	43 5	46 5	42 0	37 5	40 0
18	27 5	24 5	35 0	42 0	40 0	37 5	32 5	34 2
24	33 0	34 0	38 0	46 0	48 0	42 0	38 0	40 0
32	40 5	41 5	41 5	45 5	52 0	42 0	44 0	44 0
44	40 0	38 0	39 0	47 0	49 0	43 0	41 0	42 4
56	34 0	36 0	41 5	52 0	53 0	46 0	42 0	43 5
Mean	35 5	35 9	37 8	42 1	44 5	39 7	37 2	39 0
Biscuit B								
Initial	31 5	31 5	30 0	31 5	29 5	28 5	28 0	30 0
2	31 0	30 5	30 0	26 5	27 5	26 0	26 0	28 2
4	35 0	35 0	38 0	39 5	40 0	35 5	31 5	36 4
6	32 0	32 5	32 5	35 5	35 5	35 5	31 0	33 5
8	34 0	32 0	36 0	40 0	40 5	36 5	36 5	36 5
12	32 5	32 0	33 5	34 5	42 0	38 0	36 5	35 6
18	25 0	25 0	27 0	36 0	37 0	34 0	30 0	30 6
24	31 0	32 0	35 0	41 0	43 5	39 5	36 5	36 9
32	35 0	44 5	33 0	49 5	54 5	47 0	42 0	43 6
44	32 0	38 0	34 0	52 0	56 0	49 0	43 0	43 4
56	33 0	32 0	47 0	46 0	50 0	43 0	39 0	41 4
Mean	32 0	33 2	34 2	39 3	41 5	37 5	34 6	36 0

TABLE VII

pH VALUES OF SODIUM CHLORIDE EXTRACTS OF DEFATTED BISCUITS, STORED
AT 43.3° C. (110° F.)

Storage time, wk.	Moisture content, %							
	0	3	6	9	12	15	18	Mean
Biscuit A								
Initial	7.59	7.78	7.78	7.81	8.04	8.31	7.86	7.88
2	6.87	7.84	8.14	7.68	7.44	7.46	7.12	7.50
4	7.34	7.66	7.65	7.73	7.15	7.18	7.02	7.39
6	7.32	7.42	7.38	7.50	7.10	7.09	6.92	7.25
8	7.18	7.58	7.42	7.58	7.50	7.52	7.62	7.48
12	7.08	7.68	7.65	7.60	7.60	7.70	7.45	7.54
18	7.18	7.15	6.92	6.65	7.02	7.20	7.32	7.06
24	7.42	7.52	7.55	7.40	7.42	7.42	7.38	7.44
32	6.05	6.98	7.02	7.22	7.25	7.22	7.12	6.98
44	6.32	7.02	7.01	7.21	7.25	7.20	7.10	7.01
56	7.32	7.49	7.52	7.48	7.32	7.57	7.41	7.44
Mean	7.06	7.46	7.46	7.44	7.37	7.44	7.30	7.37
Biscuit B								
Initial	8.00	8.00	7.98	8.04	8.03	8.22	8.11	8.05
2	7.83	7.77	7.98	7.62	7.42	7.38	7.28	7.61
4	7.81	7.49	7.90	7.32	7.35	7.02	6.89	7.40
6	7.38	7.42	7.42	7.19	7.10	7.12	6.82	7.21
8	7.41	7.52	7.55	7.45	7.55	7.40	7.58	7.49
12	7.48	7.70	7.49	7.51	7.60	7.62	7.42	7.54
18	7.42	7.35	6.93	7.31	7.38	7.29	7.32	7.28
24	7.40	7.43	7.50	7.50	7.52	7.45	7.40	7.46
32	6.72	7.02	6.38	6.73	6.68	6.80	6.78	6.73
44	7.28	7.05	6.52	6.83	6.73	6.92	7.12	6.92
56	7.51	7.54	7.52	7.41	7.43	7.35	7.39	7.45
Mean	7.48	7.48	7.38	7.36	7.34	7.32	7.28	7.37

FLAVOUR REVERSION IN HYDROGENATED LINSEED OIL

II. EFFECT OF VARIATIONS IN PROCESSING PROCEDURES[1]

By H. W. Lemon[2], A. Lips[3], and W. Harold White[4]

Abstract

Shortenings prepared from various linseed oils by different methods were stored at 43.3° C. (110° F.) and sampled at eight-week intervals for 56 weeks. Storage life in terms of flavour reversion was not highly correlated with mean peroxide value or unsaturation. All shortenings were resistant to normal oxidation, but unstable to reversion unless hydrogenated to a very low iodine number. Hot and cold pressed oils yielded products equally susceptible to flavour reversion. Changes in stability attributable to variations in methods of alkali refining, bleaching, and hydrogenation were only minor. High alkali concentrations (30° to 40° Bé.) were beneficial, while hydrogenation at 190° C. (374° F.) was preferable to hardening at lower temperatures (140° C. (284° F.) and 115° C. (239° F.)). Blending with other vegetable oils, or the use of hydroquinone or a wheat-germ oil preparation as antioxidants, slightly retarded the onset of reversion. None of the laboratory or commercially prepared samples examined was considered to be a satisfactorily stable product.

In the previous paper in this series (5) data were presented which indicated that the presence of an isomeric linoleic acid, produced by hydrogenation of linolenic acid, was responsible for flavour reversion in shortenings made from linseed oil. The present paper deals with a parallel investigation, involving long-term storage studies, of the effect of various modifications in processing technique upon the susceptibility of linseed oil shortenings to reversion.

Experimental

The material selected for study consisted of hot and cold pressed raw linseed oils from representative Canadian mills; a commercial raffinate obtained by solvent segregation of refined linseed oil; refined cottonseed and sunflower seed oils; and commercial shortenings manufactured from linseed oil. Except where otherwise stated, experimental shortenings were prepared from the same batch of cold pressed raw linseed oil, according to standardized processing procedures.

Standard Processing Technique

Four and one-half times the quantity of 30° Bé. sodium hydroxide solution required for neutralization of the free fatty acids was added to raw linseed oil with stirring (80 r.p.m.) and agitation continued for 45 min. at room temperature. The rate of stirring was then reduced to 18 r.p.m. and the temperature raised as rapidly as possible to 65° C. (149° F.). Stirring was continued for 30 min., and the mixture allowed to stand for 60 min. before cooling. The decanted refined oil was filtered, using 0.75 to 1% diatomaceous earth as a filter aid. The foots were quite soft and entrained an appreciable amount of oil, causing a considerable refining loss. However, much of this oil could be separated from the foots by centrifuging. The free fatty acid content of the refined oil was of the order of 0.01% (expressed as oleic acid).

The clear refined oil was mixed with 3% of activated clay and 0.6% of activated carbon; it was agitated at 70° C. (158° F.) for 30 min. and filtered.

The bleached oil was hydrogenated in an apparatus previously described (5), at a temperature of 140° C. (284° F.) and a pressure of 25 lb. of electrolytic hydrogen. A nickel formate catalyst on a diatomaceous earth carrier, reduced in cottonseed oil with hydrogen at 265° C. (509° F.), was used in a concentration of 0.25% as nickel. The course of hardening was followed by determinations of refractive index with a Zeiss butyro-refractometer. Hydrogenation was stopped between iodine values 70 and 80. Before filtration at 100° C. (212° F.), a small amount of activated carbon was added to the oil-catalyst mixture in order to secure a clear filtrate.

Hydrogenated samples were steam-deodorized for three hours at a temperature of 200° C. (392° F.) and a pressure of 2 to 4 mm. of mercury.

Tests on Shortening Materials

Storage

Samples were stored in loosely covered glass jars at 43.3° C. (110° F.) in the absence of light, and were sampled at eight-week intervals for 56 weeks. The odour of stored samples was rated by a 10 member panel. Samples were considered to be unacceptable when five of the judges scored the material as definitely reverted. From these data the storage life in weeks was estimated statistically, except in a few cases where irregularity of the observations made graphical interpretation necessary.

Heat Test

A rapid test for reversion was employed on some of the freshly prepared materials. Samples (20 gm.) were weighed into 100 ml. beakers and heated in an oven at 200° C. (392° F.) for three one-hour periods. The odour was noted when the sample was hot, and again when it had cooled. The odour of linseed oil shortening tested in this manner is stronger and more uniform than that which develops during storage at lower temperatures, and is similar to that which occurs in baked products containing linseed oil shortening. This intensification of odour may be due to oxidation or other further decomposition

caused by the heat treatment. All hydrogenated linseed oil samples except those having very low iodine numbers developed this odour. Since the differences observed among samples in the shortening range (iodine value 65 to 80) were not great, regardless of the method of processing, this test was used only in a qualitative way.

Baking

With the co-operation of Mr. A. G. O. Whiteside of the Cereal Division, Dominion Central Experimental Farm, Ottawa, a number of linseed oil shortenings were tested in bread at a level of 4% of the weight of the flour. Both the bread and toast prepared from it were tasted by panels. Differences among bread samples containing various linseed oil shortenings were not significant, even with shortenings which differed markedly by the rapid heat and storage tests. It was thought that toasting might accentuate any differences in the stability of the shortenings, but this was not observed to be the case. Hence, the bread baking test was considered to be of little value in assessing relative resistance to flavour reversion of linseed oil shortenings.

Chemical Determinations

Peroxide oxygen at each sampling time was determined by a modification of Lea's method (3). Saturated and iso-oleic acids were estimated by the lead salt precipitation method of Baughman and Jamieson (2). For iodine numbers Wij's method (4) was employed.

As none of the chemical measurements was correlated highly with storage life as estimated in terms of resistance to flavour reversion (Table I) these data are not presented in detail. In all linseed oil shortening samples, flavour reversion was well advanced before peroxide value showed any marked increase. Storage life and iodine value were not significantly correlated, but there was some indication that stability to flavour reversion might decrease

TABLE I

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN MEASURE-
MENTS ON 36 LINSEED SHORTENINGS

Quantities correlated	r
Storage life with:	
Mean peroxide oxygen value	.13
Iodine number	−.35
Saturated acids content	.05
Iso-oleic acid content	.39*
Iodine number with:	
Mean peroxide oxygen value	.05
Saturated acids content	−.86**
Iso-oleic acid content	−.63**

* Indicates 5% level of significance.
** Indicates 1% level of significance.

with increasing unsaturation. Storage life was significantly related to iso-oleic acid content. As expected, iodine value correlated highly with both saturated and iso-oleic acids contents.

Variations in Processing Procedures

The estimated storage lives, together with the calculated necessary differences, for shortening samples prepared by various processing techniques are presented in Table II.

Hot and Cold Pressed Oils

Samples of six hot and seven cold pressed oils from four companies were treated by the standard procedures outlined above for refining, bleaching, hydrogenation, and deodorization.

Storage tests (Table II (*A*)) revealed that hot and cold pressed oils yielded products equally resistant to flavour reversion; i.e., the six hot pressed oils had an average keeping time of 33 weeks, the seven cold pressed, 32 weeks. No definite differences could be detected by means of the rapid heat test.

Effect of Sodium Hydroxide Concentration in Refining

Cold pressed linseed oil was refined with 5°, 10°, 20°, 30°, and 40° Bé. sodium hydroxide solutions. The amount of sodium hydroxide in proportion to the weight of oil was kept constant, so that the only variable was the water content. Attempts were also made to refine raw linseed oil with saturated sodium hydroxide solution and with sodium hydroxide flakes. The refined oils were bleached, hydrogenated, and deodorized.

Reference to Table II (*B*) indicates that the use of the higher alkali concentrations in refining tended to retard the onset of flavour reversion in the hardened material. This result was partially confirmed by the rapid heat test, where five out of seven persons considered the product from oil refined with 40° Bé. lye to be superior to that treated with 5° Bé. lye.

When a saturated sodium hydroxide solution was used, the resulting refined oil was dark in colour, and foamed badly during hydrogenation and deodorizing. With sodium hydroxide flakes, the oil was not neutralized unless violent agitation at about 70° C. (158° F.) was employed. This procedure caused darkening of the oil. Furthermore, the soap was so finely dispersed that its separation from the oil was very difficult. It was concluded that it was impractical to use sodium hydroxide solutions stronger than 40° Bé.

Bleaching

One series of cold pressed oils, refined with 30° Bé. lye, was bleached with 1, 3, and 5% of activated clay, and another series with the same concentrations of activated carbon. Each oil was hydrogenated and deodorized in the usual manner. An unbleached sample was processed at the same time as a control.

Table II (*C*) shows that the use of less clay and more carbon in the standard bleaching process should be beneficial.

TABLE II

STORAGE LIFE AT 43.3° C. (110° F.) OF LINSEED SHORTENINGS PREPARED BY VARIOUS PRO-
CESSING PROCEDURES, IN TERMS OF WEEKS BEFORE THE APPEARANCE
OF DEFINITE REVERSION

Group	Group variant	Storage life, weeks
A. Hot and cold pressed linseed oils	Hot pressed	27
	Hot pressed	39
	Hot pressed	16
	Hot pressed	31
	Hot pressed	45
	Hot pressed	42
	Cold pressed	29
	Cold pressed	30
	Cold pressed	34
	Cold pressed	34
	Cold pressed	32
	Cold pressed	38
	Cold pressed	33
B. Effect of concentration of sodium hydroxide solutions in refining	5° Bé.	25
	10° Bé.	35
	20° Bé.	40
	30° Bé.	50
	40° Bé.	42
C. Effect of bleaching agents	Unbleached	31
	1% clay	29
	3% clay	35
	5% clay	0
	1% carbon	37
	3% carbon	33
	5% carbon	42
D. Hydrogenation to various iodine values	161.6	0
	133.5	9
	92.0	26
	57.3	15
	38.1	24
	6.1	60 +
E. Catalysts, at different temperatures	Commercial *A*, 115° C.	38
	140° C.	36
	190° C.	57
	Commercial *B*, 115° C.	47
	140° C.	42
	190° C.	53
	Commercial *C*, 115° C.	52
	190° C.	58
	Commercial *D*, 140° C.	11
	190° C.	5
	Oil-reduced formate, 115° C.	31
	140° C.	39
	190° C.	43
	Dry-reduced carbonate, 115° C.	33
	190° C.	42

TABLE II—*Concluded*

STORAGE LIFE AT 43.3° C. (110° F.) OF LINSEED SHORTENINGS PREPARED BY VARIOUS PRO-
CESSING PROCEDURES, IN TERMS OF WEEKS BEFORE THE APPEARANCE
OF DEFINITE REVERSION—*Concluded*

Group	Group variant	Storage life, weeks
F. Blends with sunflower seed oil	100% linseed	27
	75% linseed	24
	50% linseed	27
	25% linseed	27
	0% linseed	28 (rancid)
G. Antioxidants	Formula C, 0.1%, before hydrogenation, hot pressed oil	38
	Formula C, 0.1%, before hydrogenation, cold pressed oil	26
	Formula C, 0.1%, before bleaching	23
	Formula C, 0.1%, before hydrogenation	21
	Formula C, 0.1%, before deodorizing	26
	Formula C, 0.1%, after deodorizing	45
	Hydroquinone, 0.02%, before bleaching	23
	Hydroquinone, 0.02%, before hydro-genation	27
	Hydroquinone, 0.02%, before deodorizing	26
H. Linseed oil raffinate	Raffinate, unbleached	31
	Raffinate, bleached	35
	Raffinate, hydrogenated by a commercial firm	41
Necessary difference	For storage life 0 to 35 weeks	10
	For storage life 35 to 60 weeks	16

Hydrogenation to Various Iodine Values

Refined and bleached cold pressed oil was hydrogenated to the following iodine values: 162, 134, 92, 57, 38, and 6. All the products and also a sample of unhydrogenated oil were deodorized.

By the use of the rapid test it was found that the unhydrogenated oil and the sample having an iodine value of 162 both developed a strong "painty" odour on heating. At an iodine value of 134 the painty odour was almost gone, but the characteristic reversion odour could be detected. The reversion odour was at its maximum at iodine value 92, definitely less at 57, almost absent at 38, and definitely gone at iodine value 6. It has been pointed out that the intensity of this odour roughly parallels the accumulation and disappearance of iso-linoleic acid (5).

The storage data presented in Table II (D) in general corroborate the observations of the rapid test. However, the differences for the middle range of iodine values are not significant.

Hydrogenation with Various Catalysts at High and Low Temperatures

Four commercial and two laboratory prepared nickel catalysts were tested. The preparation of oil-reduced nickel formate has been described in the section on standard methods. Dry-reduced nickel carbonate was prepared as follows. Nickel nitrate was dissolved in water, and a quantity of diatomaceous earth added. Sodium carbonate solution was added slowly and in excess while the mixture was stirred. The precipitate was collected by filtration, washed with water, and dried. The dry powder was reduced at 400° C. (752° F.) for three hours in an atmosphere of hydrogen. When cool, it was quickly transferred to melted shortening. The nickel content of each catalyst was determined, and quantities equivalent to 0.1% nickel on the weight of the oil were employed. The catalysts were tested at 115°, 140°, and 190° C. (239°, 284°, and 374° F.).

According to the storage (Table II (E)) and rapid heat tests, only one of the six catalysts studied, commercial sample D, was definitely unsuitable; however, this catalyst was not intended for hydrogenation of oils. Of the other catalysts, the commercial products appeared to yield more stable shortenings than did the laboratory prepared materials. These differences were significant in several cases. Hydrogenation at 190° C. (374° F.) produced shortenings with longer storage lives as compared to hydrogenation at the lower temperatures. This increase was significant only in the case of commercial catalyst A, but a general trend towards increased stability with higher hydrogenation temperatures was observed.

Blends

Refined and bleached cold pressed linseed oil and refined sunflower seed oil were used in the preparation of a series of pure and blended oils, as follows: (i) 100% of linseed oil, (ii) 75% of linseed oil, 25% of sunflower seed oil, (iii) 50% of linseed oil, 50% of sunflower seed oil, (iv) 25% of linseed oil, 75% of sunflower seed oil, (v) 100% of sunflower seed oil. A similar series of blends of linseed oil with cotton seed oil was prepared. Each oil, or combination of oils, was hydrogenated and deodorized in the usual manner.

In rapid heat tests, as expected, the typical odour of reversion decreased as the proportion of sunflower oil was increased. However, the storage tests (Table II (F)) did not reveal any significant differences, as the hydrogenated sunflower seed oil component was no more resistant to rancidity than the linseed shortening itself was resistant to flavour reversion. Similar results were obtained with the series of cottonseed oil blends, for which storage lives are not reported.

Use of Antioxidants

Two antioxidants were used: Formula C, a wheat-germ oil preparation (6), in a concentration of 0.1%; and hydroquinone, in a concentration of 0.02%.

These were added (*a*) before bleaching, (*b*) before hydrogenation, (*c*) before deodorizing, and (*d*) after deodorizing. Formula *C* was also tested in shortenings made from samples of hot and cold pressed oils which were refined by means of a special silica-gel–alumina adsorbent (1).

Table II (*G*) shows that the only instance in which the added antioxidant significantly increased the storage life over the average for standard processing (32 to 33 weeks) was with formula *C* after deodorizing. This was confirmed by rapid heat tests.

Commercial Products

A commercial linseed oil raffinate, obtained by solvent segregation of refined linseed oil, was hydrogenated and deodorized in the standard manner. A shortening prepared from this oil by a commercial firm was included in the tests. Two other commercial shortenings purported to be made from a 100% linseed oil base were also examined.

The commercial raffinate had a lower iodine number than any of the linseed oils used, and therefore required less hydrogenation. However, the product, when heated to 200° C. (392° F.), developed the same unpleasant odour that is characteristic of hydrogenated linseed oil, and the storage life was no better than that of the linseed oil shortenings prepared in the laboratory. A shortening prepared from this oil by a commercial firm had a somewhat longer storage life (Table II (*H*)).

The two commercial linseed oil shortenings developed the usual reverted odour in the rapid heat test. Bread baking tests at the Dominion Central Experimental Farm confirmed this finding. No storage data were obtained.

Discussion

The results of these studies indicate that variations in the usual fat processing techniques do not yield a satisfactory solution to the problem of flavour reversion in linseed oil shortening. The product reverted readily when heated to baking or frying temperatures unless it had been hydrogenated to a very low iodine number. When it was stored at lower temperatures, differences in stability induced by changes in treatment were more readily estimated, but most of these differences were not significant.

Some slight improvements in stability may be obtained by modifications of the customary processing procedures, but it appears that the difficulty of flavour reversion in linseed oil shortening should be attacked at its source, the tri-unsaturated components of the linseed oil, in line with the iso-linoleic acid formation theory already developed (5). Separation of a major portion of the linolenic glycerides from the oil prior to hydrogenation, or a more selective hydrogenation to minimize the formation of iso-linoleic constituents, would seem to be indicated.

Acknowledgments

References

1. ARMSTRONG, J. G. and McFARLANE, W. D. Oil & Soap, 21 : 322-327. 1944.
2. BAUGHMAN, W. F. AND JAMIESON, G. S. Oil & Fat Industries, 7 : 331-332. 1930.
3. FRENCH, R. B., OLCOTT, H. S., and MATTILL, H. A. Ind. Eng. Chem. 27 : 724-728. 1935.
4. JAMIESON, G. S. Vegetable fats and oils. Reinhold Publishing Corp., New York. 1943.
5. LEMON, H. W. Can. J. Research, F, 22 : 191-198. 1944.
6. LIPS, A. and McFARLANE, W. D. Oil & Soap, 20 : 193-196. 1943.

A SIMPLE METHOD OF SEALING GAS- OR VACUUM-PACKED TINS

By W. A. Bryce and H. Tessier

A SIMPLE METHOD OF SEALING GAS- OR VACUUM-PACKED TINS[1]

By W. A. Bryce[2] and H. Tessier[3]

Abstract

A method of soldering tins in a gaseous atmosphere or in a vacuum is described. The heating element is a coil of resistance wire supported over a hole in a flat surface of the tin. When the heating circuit is closed, a small piece of solder previously hung in the upper end of the coil is melted and drops on the area about the hole and thereby produces an effective seal.

Introduction

The need for a method of sealing gas- or vacuum-packed tins arose in this laboratory in connection with studies being conducted on the storage of dehydrated foods. Previous investigators (3) have used glass containers for vacuum packing. Gas packing of egg powders has been done by soldering the containers immediately upon removal from the gas chamber, assuming that the sorption of carbon dioxide or nitrogen was slow enough to permit sealing without oxygen intake. However, further investigations (2) have shown that the sorption of oxygen by milk powders is sufficiently rapid to make the above method of questionable value.

A method (1) of sealing tins by means of a soldering iron extending into a desiccator was found to be slow and cumbersome. An effort was therefore made to devise a faster and more convenient technique. This paper describes the method developed for sealing tins in either gas or vacuum.

Description of Apparatus

The method devised employed a soldering technique using a small coil of resistance wire as a heating element. A sealing unit is shown in Fig. 2.

The coil was made from six inches of No. 18 chromel C wire and had two half-inch turns in the lower part and six smaller turns in the upper. When the coil was in position the lowest turn rested on the flat surface of the tin. The coil was connected to the external circuit by two binding posts mounted on a bakelite platform. The platform was supported by a metal extension attached to one side of a small battery clip, the jaws of which had been shaped to fit the contour of the protruding rim of the container, thus holding the apparatus in position.

[2] *Biochemist, Food Investigations,*

[3] *Laboratory Steward, Food Investigations.*
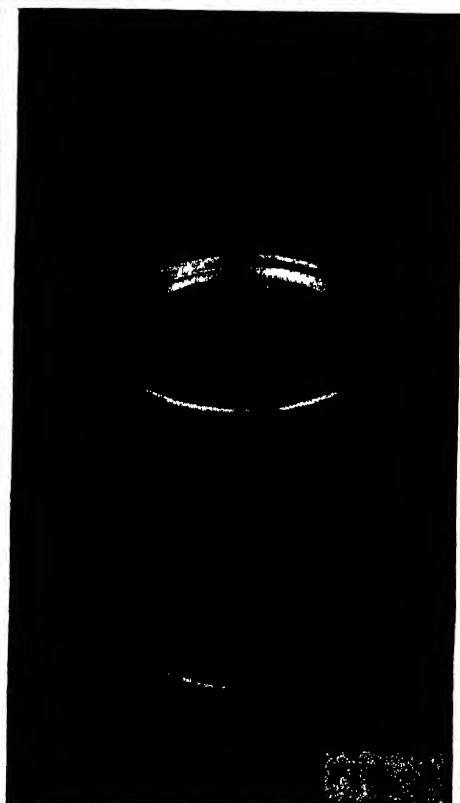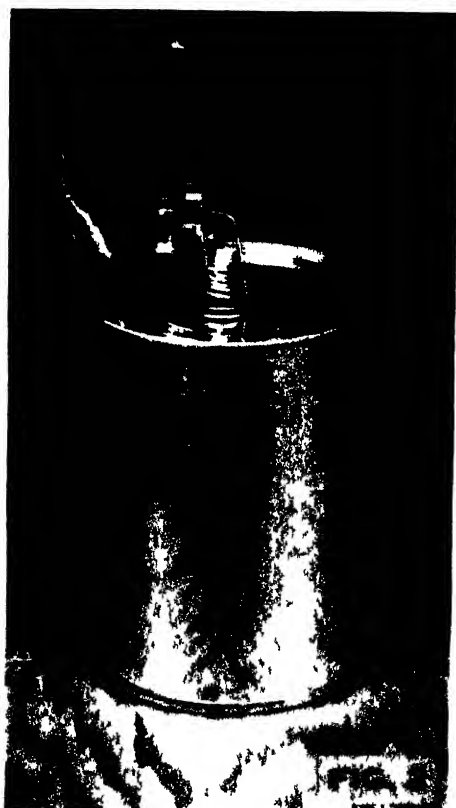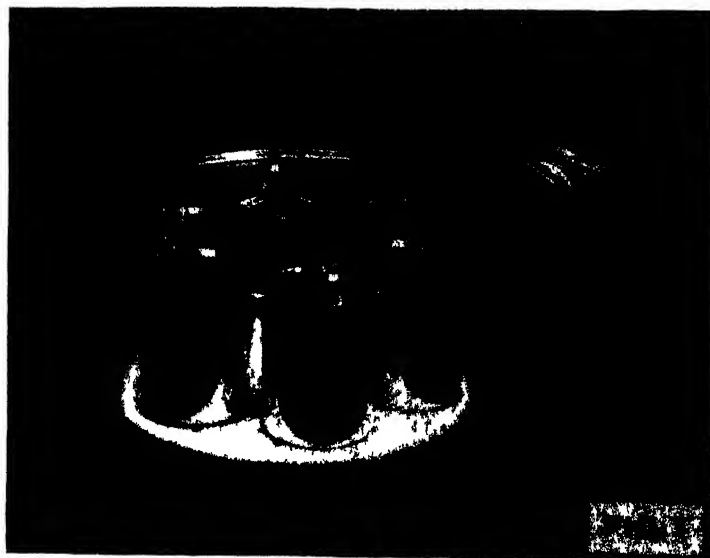
PLATE I

FIG 1   Tins with soldering devices attached in position in a vacuum desiccator
FIG 2   The soldering apparatus in position on a tin
FIG 3   An example of the seal obtained under vacuum

Procedure

The material under investigation was canned, using a suitable canning machine. A small hole, approximately $\frac{1}{16}$ in. in diameter, was then made in the centre of one end of the tins. The surface of the tins was lightly etched with sandpaper to obtain better soldering conditions. A suitable number of tins, each with its own soldering coil, was placed in a vacuum desiccator containing a porcelain plate as a supporting base. The tins were insulated from each other. The soldering units were then connected in series, the power leads passing out of the desiccator through the rubber stopper supporting the air-outlet stopcock. A $\frac{1}{4}$ in. length of acid core wire solder was placed in the upper end of each coil, one end of the solder 'bullet' being flattened to prevent it from dropping through the coil. Fig. 1 shows the assembly with the tins and soldering units arranged and the desiccator ready for sealing.

When the desired vacuum or gas composition was attained, the stopcock was closed and a potential applied across the external power leads, causing the solder bullets to melt, drop down, and seal the holes in the tins. The surface of the tins around the holes had been heated by contact with the lowest turn of the coil, thus ensuring proper conditions for soldering. After the few seconds required to melt the solder and seal the tins, the heating circuit was opened, the pressure brought to atmospheric, and the sealed tins were removed.

The entire operation can be completed in half an hour. An example of the type of seal obtained is shown in Fig. 3.

Discussion

The simplicity of the method makes it readily applicable to laboratory studies. The limit to the number of tins that can be sealed simultaneously obviously depends on the dimensions of the vacuum chamber, the size of the tins, and the power source available. The apparatus described above may be used on larger tins by making suitable alterations in design.

References

1. DOAN, F. J. and JOSEPHSON, D. V. Food Industries, 16 (5) : 91-92. 136. 1944.
2. PEARCE, J. A. * In press.
3. WHITE, W. H., THISTLE, M. W., and REID, M. Can. J. Research, D, 21 : 271-276. 1943.

DRIED MILK POWDER

II. FACTORS AFFECTING THE SORPTION OF CARBON DIOXIDE[1]

By Jesse A. Pearce[2]

Abstract

Sorption of carbon dioxide by milk powder in a closed system at 35° C. and at approximately 74 cm. of mercury was observed to be greater than 0 4 cc. per gm. after 150 hr., while only 0.012 cc. of nitrogen was absorbed per gm. after 70 hr. The initial sorption of carbon dioxide varied with time according to the equation:

$$s^m = kt$$

where s is 100 times the amount sorbed in cc. per gm. at any time, t (min.), and k and m are constants peculiar to the system under investigation. The logarithmic form of this equation was used.   Powders with 26, 28, and 30% fat did not differ in behaviour, but sorption curves for powders with only 1% fat had lower $\frac{\log k}{m}$ values and lower $\frac{1}{m}$ values than the curves for the high fat levels. Powders with 1% fat sorbed carbon dioxide in an identical manner when exposed to either 100% carbon dioxide or a mixture of 20% carbon dioxide and 80% nitrogen. For whole milk powder, dilution to 80% nitrogen content was effective in reducing the initial sorption rate of carbon dioxide.   Great variation was observed in the sorption behaviour of powders from different plants and in powders produced at different time intervals in the same plant.   Temperature differences within the range 25° to 40° C. had no effect on sorption.   Palatability and $\frac{1}{m}$ correlated to the extent of $r = .61$.

Introduction

Packing milk powder in an atmosphere of carbon dioxide or in mixed nitrogen and carbon dioxide has become common commercial practice.   The use of nitrogen has been studied, but has been reported to be unfavourable (7).   Carbon dioxide is believed to react with the fat (2) and its use appears to add little to the storage life of milk powder (2, 7, 8).   In spite of this, carbon dioxide is still being used (3).   During the development of a cellulose base container for gas packing (8), data on the sorption of carbon dioxide by milk powder were required, but little could be found in the literature.   Therefore, a study of this problem was believed desirable, particularly if milk powder were to be packed in such a flexible container.   The present paper records the effect of gas composition (carbon dioxide and carbon-dioxide–nitrogen mixtures), temperature, moisture content, fat content, and variations in processing on the sorption of carbon dioxide by milk powder.

Materials

Currently available, spray dried milk powders from Canadian plants were used. Since the product deteriorates rapidly, fresh materials were obtained for each phase of the study. The moisture and fat contents are noted in Fig. 3. These powders were used without further treatment, except that, in the study of moisture effects, moisture contents were adjusted to levels between 1.8 and 5.0%.

Sorption Method

Preliminary measurements on two sample tins of commercially available milk powder showed that there was 0.57 gm. of milk solids to every cubic centimetre of container space. However, the solids occupied only 0.26 cc. of container space. The remainder must have been headspace and interstitial space, plus the space inside the hollow, spherical milk particles (5). The inclusion of this last item seems permissible since the packing density averaged about 0.86 gm. per cc. of powder.

The apparatus, shown diagrammatically in Fig. 1, permitted measurement of sorption rates at 0 3 gm. of milk solids per cubic centimetre of container space, as opposed to the value of approximately 0 6 gm. per cc. of commerical container space. Since the initial sorption rate is not dependent on container volume, it was believed that this apparatus would give information of value.
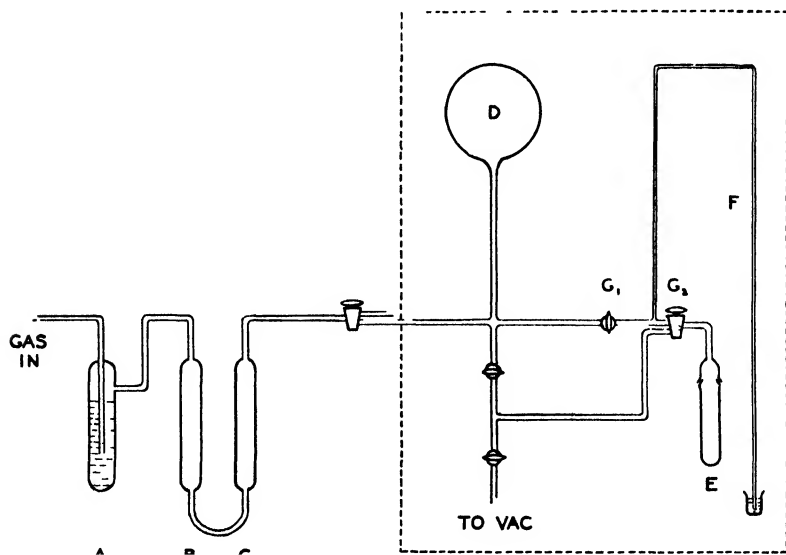

FIG. 1. *Diagram of sorption apparatus.*

Carbon dioxide or nitrogen was passed through the purifying train shown at *A*, *B*, and *C*, which represent alkaline pyrogallol (to remove oxygen), calcium chloride, and phosphorous pentoxide (both to remove water vapour). The gas or gas mixture was then stored in the five litre volume (*D*),which

permitted flooding of the bulb (*E*) without reducing the pressure in the sorption system by more than 1.5 cm. of mercury.

The sorption bulb (*E*) was filled with 50 gm. of milk powder and together with the manometer (*F*) evacuated through G_2 for 15 min. After evacuation the stopcock (G_1) was opened momentarily to permit pressure equalization between bulbs *D* and *E*. The pressure decreases in the system during a five-hour period were determined at logarithmic time intervals and were used to calculate the amount of gas sorbed by the milk powder.

The dotted lines indicate the portion of the apparatus enclosed in an air jacket that could be controlled at the desired temperatures (25° to 40° C.) to ± 1° C. Average barometric pressure throughout the experiment was 75.7 with a range of 74.7 to 77.2 cm. of mercury. Since the expansion of gas from bulb *D* into bulb *E* reduced the pressure 1 5 cm., the average initial pressure in the system was approximately 74 cm. of mercury (Fig. 1).

In a portion of the study, palatability scores were determined by a method described previously (6). Panels of 14 tasters scored the reconstituted milk on the basis of 10 (the equivalent of whole milk) to 0 (a repulsive sample).

Results

Interpretation of Results

The curves shown in Fig. 2A depict sorption against time at 35° C. with the pressure initially at 74 cm. of mercury. Sorption is expressed as 100 times the volume of gas sorbed by a gram of milk powder. These curves indicate that carbon dioxide was extensively sorbed, while nitrogen was sorbed only to a minor extent, and that dilution of the carbon dioxide with nitrogen markedly altered the sorption curve. The amount of carbon dioxide sorbed after 150 hr. was 0.444 cc. per gm. After 70 hr. only 0.012 cc. of nitrogen was sorbed per gm. The logarithmic curve for sorption by nitrogen seems to indicate a lengthy induction period followed by an increased sorption rate. It was observed that the amount sorbed varied with time according to the equation:

$$s^m = kt,$$

where *s* is the amount sorbed per gm. at any time, *t*, in minutes and *k* and *m* are constants. This relation has been previously observed for the sorption of gases on metals, glass, etc. (1, pp. 18-20; 4). Verification of the relation for milk powder was sought by the plotting of logarithmic curves as shown in Figs. 2B, 2C, where log *s* is plotted against log *t*.

As in previous observations (1, 4) the experimental points deviated from the straight line as saturation of the sorbent was approached, or as the concentration of gas in the system decreased. As a result, in a system containing 20% carbon dioxide and 80% nitrogen, the logarithmic relation had to be determined by the sorption occurring in about the first hour. Otherwise the relation was based on the sorption curve for a five hour period.

For convenience in handling the data, values were transposed to logarithms and $\log k$ and $\frac{1}{m}$ were calculated from the equation:

$$\log s = \frac{\log k}{m} + \frac{1}{m} \log t$$
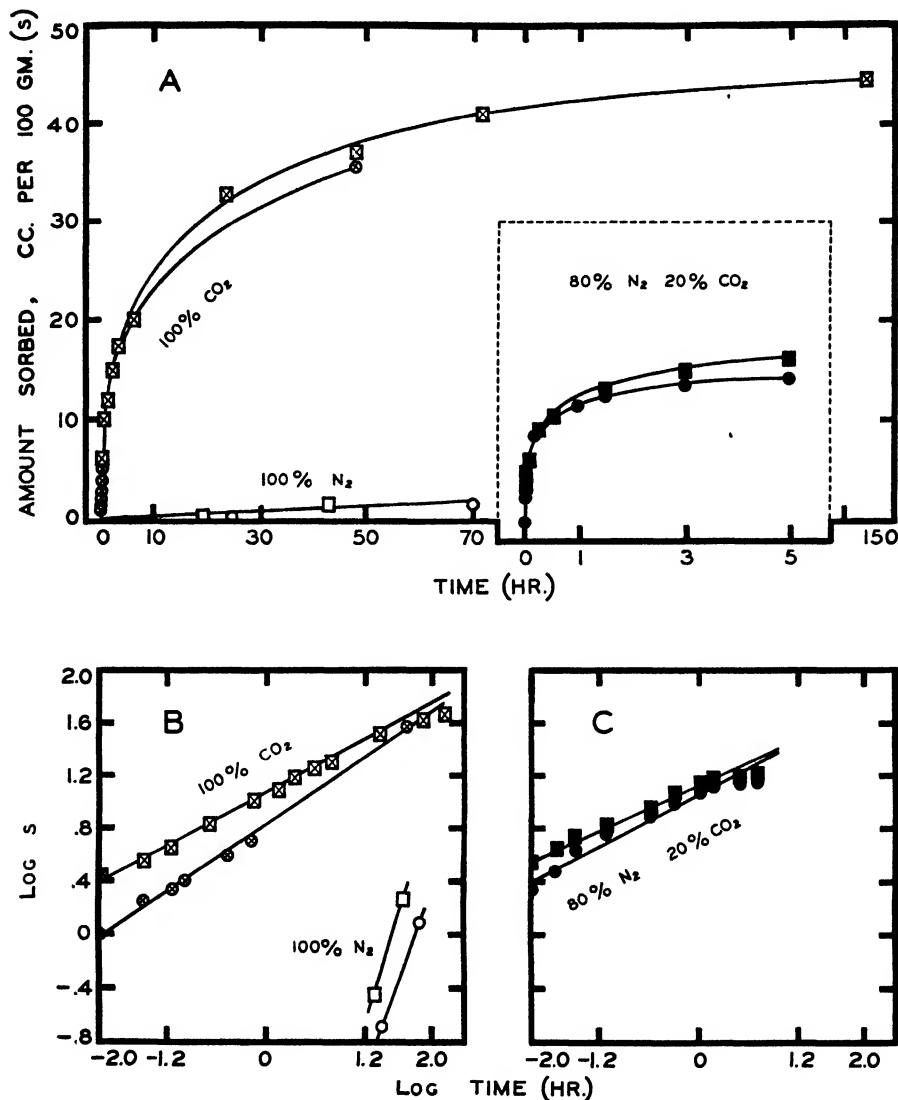
FIG. 2.   *Sorption of carbon dioxide and nitrogen by milk powder.*

Differences between values in $\log k$ and $\frac{1}{m}$ were selected either by analyses of variance or by visual inspection.   All results differing significantly were then shown as curves of $\log s$ against $\log t$.

Duplicate curves were established for a sample from each of two plants. The results, in Fig. 3A, show that by means of this apparatus it was possible to obtain good agreement between duplicates. Therefore, in subsequent work only a single curve was determined for each sample.
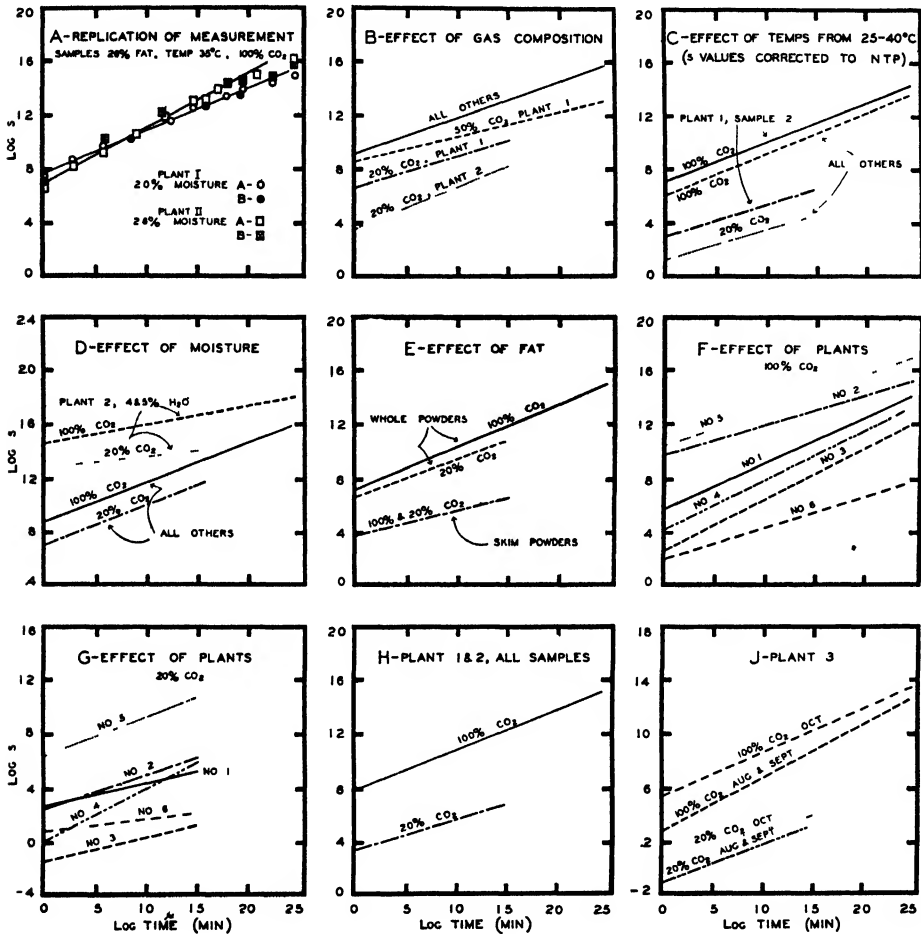
FIG. 3. *Effect of various factors on the sorption of carbon dioxide by milk powder.*

In general, samples of whole milk contained from about 2 0 to 2 5% moisture and 26% butterfat, while samples of skim milk contained about 3% moisture and 1% butterfat. Gases were carbon dioxide or carbon dioxide and nitrogen.

B. *"All others" describes samples from Plant 1 in an atmosphere of 100 and 80% carbon dioxide, and samples from Plant 2 in an atmosphere of 100, 80, and 50% carbon dioxide.*

C. *"All others" describes samples from each of Plants 1 and 2.*

D. *"All others" describes samples of 1 8, 2 3, 3 0, 4.0, and 5 0% moisture from Plant 1, and samples of 1 8, 2.3, and 3 0% moisture from Plant 2.*

E. *"Whole powders" describes samples of 26 and 28% butterfat from Plant 1 and samples of 26, 28, and 30% butterfat from Plant 2.*

"Skim powders" describes samples of 1% butterfat from both Plants 1 and 2.

The Effect of Temperature and Partial Pressure of Carbon Dioxide

The effect of diluting carbon dioxide with nitrogen in powders containing 26% fat from two plants is shown in Fig. 3B.   Dilution with 80% nitrogen was necessary to reduce effectively the rate of sorption of carbon dioxide by milk powder.   Further comparisons were made only between the effects of sorption from 100% carbon dioxide and from a mixture of 20% carbon dioxide and 80% nitrogen.

Temperature effects between 25° and 40° C. were negligible when the amount of gas sorbed was calculated to N. T. P. (Fig. 3C).   Again some differences between samples were evident and differences between concentrated and diluted carbon dioxide were marked.

Effect of Moisture Content and Fat Content

Powders containing 26% fat from two plants were adjusted to moisture levels between 1.8 and 5.0%.   Milk powders from one plant at 4 and 5% moisture showed alteration in the shape of the sorption curve (Fig. 3D). All other samples had normal sorption curves.   It is possible that a layer of surface water was formed on these abnormal samples and dissolved some carbon dioxide.

Milk powders with fat contents of 26, 28, and 30% did not differ in either slope or constant in the logarithmic equation (Fig. 3E).   However, reduction of the fat content to 1% had a significant effect and resulted in common behaviour for both samples at both carbon dioxide levels.   It is apparent that carbon dioxide is sorbed not only in the butter fat, but by the other constituents of milk powder.

Differences Between and Within Plants

Further information concerning this variation observed in material produced by different plants was believed desirable.   The curves in Figs. 3F and 3G show that variations in products from six plants far exceeded any of the differences observed up to this point, and show little consistency in behaviour of powders from different sources.   It is also evident from Fig. 3H and 3J that considerable variation is likely to occur in products from the same source.

Relation Between Sorption and Palatability

It was of interest to evaluate the relation between constants of the logarithmic equation and the palatability of the powders.   The correlations have been determined as follows: palatability and log k, $r = -.26$; palatability and $\dfrac{\log k}{m}$, $r = .07$; palatability and $\dfrac{1}{m}$, $r = .61^*$.   This correlation is higher than any observed previously for objective measurements against palatability (6).

Although there is a tendency for the slope of the curve expressing the logarithmic relation between s and t to relate to palatability, the use of sorption

* *Surpasses 5% level of statistical significance.*

technique as a means of determining quality is usually too cumbersome to be of practical significance (6). However, the results indicate that sorption of carbon dioxide bears some relation to milk powder structure as affected by drying practice and evident in the palatability of the reconstituted product. Attention is being given to possible use of this technique as a quantitive measure of milk powder quality.

Acknowledgments

References

1. GREGG, S. J. The adsorption of gases by solids. Methuen and Co. Ltd., London. 1934.
2. HOLM, G. E., WRIGHT, P. A., and GREENBANK, G. R. J. Dairy Sci. 10 : 33-40. 1927.
3. LEA C. H., MORAN, T., and SMITH, J. A. B. J. Dairy Research, 13 : 162-215. 1943.
4. MCBAIN, J. W. Sorption of gases and vapours by solids. George Routledge and Sons, Ltd., London. 1932.
5. PALMER, L. S. and DAHLE, C. D. J. Dairy Sci. 5 : 240-245. 1922.
6. PEARCE, J. A. Can. J. Research, F, 23 : 177-184. 1945.
7. SCHATZEL and CLAUSEN. Molkerei-Ztg. 53 : 1532-1534. 1939. *Abstracted in* Chem. Abstracts, 34 : 4169². 1940.
8. WOODCOCK, A. H. Can. J. Research, F, 23 : 117-122. 1945.

DRIED MILK POWDER

III. THE EFFECT OF LIGHT ON KEEPING QUALITY

By Jesse A. Pearce and W. A. Bryce

DRIED MILK POWDER

III. THE EFFECT OF LIGHT ON KEEPING QUALITY[1]

BY JESSE A. PEARCE[2] AND W. A. BRYCE[2]

Abstract

At an average temperature of 6° C., exposure of both whole and skim milk powders to sunlight caused more rapid deterioration in quality than occurred in the dark. Ultra-violet light with a principal wave length of 3800 Å accelerated deterioration in whole milk powder stored at 38° C., but had no significant effect on skim milk powders; the effect of this light on whole milk powders was less pronounced than that produced by sunlight. Storage of samples at 38° C. under different light intensities indicated that the differences between ultra-violet and sunlight were the result of the difference in total energy of light falling upon the sample, rather than the difference in wave length of the activating light.

Introduction

One of the typical off-flavours known to develop in milk powder has been termed 'sunlight-taint.' Included in sunlight taint are other off-flavours developed as a result of exposure to bright artificial light (2, p. 437). The effect of light on numerous fats has been studied (3, pp. 139-152), but little definite information about the effect of sunlight on milk powder is available. The present paper describes an experiment designed to obtain some information about the effect of light on spray-dried milk powder of different fat levels and from different sources.

Materials and Methods

The milk powders used were the commercially available products of two Canadian companies. Plant X produced materials having 1, 26, 28, and 30% fat, and Plant Y produced products with 1, 26, and 28% fat. The skim milk powders (1% fat) had a moisture content of about 3%, while the whole milk powder had moisture contents of between 2.0 and 2.5%.

After the powders were tested for initial palatability, they were divided into four equal portions. One portion was subjected to outdoor sunlight for a period of 48 days, during which there was 215 hr. of sunlight. The average temperature during this period was 6° C. (43° F.). The 'light' energy falling on the samples was calculated as approximately 1.8 cal./sq. cm./min. A comparable set of samples in light-proofed containers was stored under the same conditions.

The third set was stored in the laboratory in proximity to an ultra-violet lamp for the periods shown in Fig. 1. The maximum light transmitted by this lamp was at a wave length of 3800 Å. and the range of ultra-violet produced was from 3200 to 4200 Å. The storage temperature in this phase

of the experiment was 38° C. (100° F.). The calculated light energy falling on the sample was approximately 0.0034 cal./sq. cm./min. The fourth set of samples was stored in light-proofed containers at the same temperature to serve as controls.

An investigation of the effects of light intensity, done in duplicate, utilized only powder from one company (fat content of 1 and 26% and moisture content approximately 2.5%). The samples were spread in thin layers on large watch glasses and were protected from moisture change. Samples in both light-proofed and untreated watch glasses were stored (38° C.) at distances of one and four feet from a 100-watt incandescent lamp. The light intensities falling on the samples at the one-foot and four-foot levels were 0.0036 and 0.0004 cal./sq. cm./min., respectively. These materials were sampled for quality after three days (75 hr. exposure to light).

In all phases of this investigation the headspace gas in the containers was air.

The palatability of reconstituted samples was determined by the method used in these laboratories (6). Scoring was done on a scale from 10 to 0, 10 being the equivalent of excellent fresh whole or skim milk.

Results

The Effect of Light on Quality Changes During Storage

The results, using dry whole milk from two sources, and at different fat levels, were assessed by an analysis of variance. The effects shown to be significant are recorded in Fig. 1. In general, no differences in rate of deterioration were observed between powders from different sources or between powders
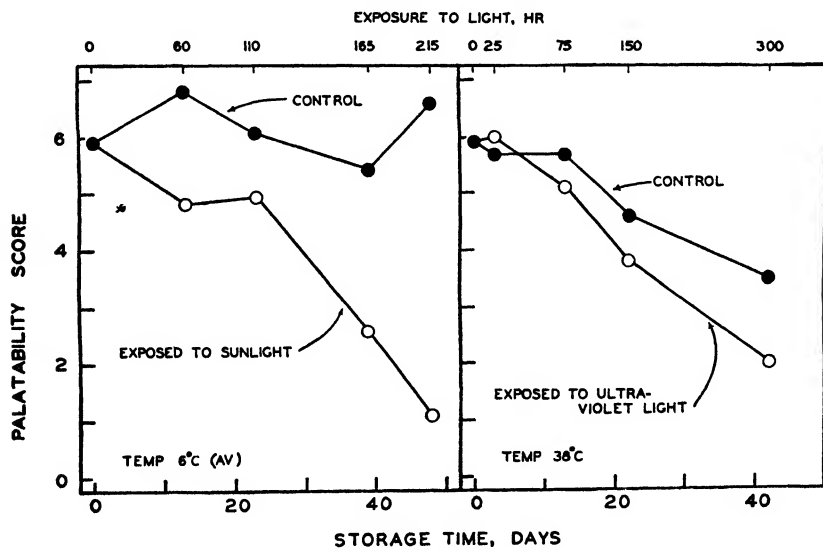

FIG. 1. *The effect of sunlight and ultra-violet light on the deterioration of spray-dried whole milk powder.*

with different fat levels. However, the 26% fat sample from Plant X appeared to be more light-sensitive than the others. The control samples held at 6° C. suffered no deterioration during the storage period, while control samples held at 38° C. deteriorated in a manner comparable to that noted earlier (5, 7).

The samples exposed to sunlight deteriorated much more rapidly than the control samples (Fig. 1). The samples subjected to 215 hr. of sunlight decreased about 4.8 palatability units. From the shape of the curves it is evident that the initial period of exposure to sunlight had little effect on palatability but the sunlight effects became more pronounced with increased exposure. Increased temperature accelerated the deterioration of these products (1). Since temperature and light effects are likely to be additive (3, pp. 139-152), it might be expected that sunlight effects would become more marked as the temperature increased. For these reasons, it is recommended that powders be exposed to sunlight for no longer than a five-hour period.

The difference in palatability between controls and samples exposed to the ultra-violet lamp was less marked than the differences between controls and samples exposed to sunlight. Exposure to 150 hr. illumination from this source decreased storage life by about 10 days, and after 300 hr. the difference between control and exposed powders was only 1.5 palatability units. Two factors may have been responsible for this effect. The energy for the ultra-violet lamp was extremely low as compared with sunlight, and in addition, the wave lengths emitted may not have been those causing maximum deterioration.

The skim milk powders (Table I) behaved in a manner similar to that previously noted (5), i.e., after a short period in storage the palatability seemed somewhat improved, suggesting that volatile degradation products formed in the powder during the dehydration process decreased during storage. This may not be noticeable in whole milk powders since fat is deteriorating simultaneously, the over-all result being lower palatability. Dried pork showed similar improvement in quality during the initial storage periods (4).

Only two effects of significance (Table I) were noted in these studies on skim milk. Powder from Source Y was generally of lower palatability than powder from Source X. This difference attained significance at the higher temperature. At both the combinations of temperature and light, the control samples received higher palatability scores than the exposed samples, but the difference was significant only in that portion of the experiment where powders were exposed to sunlight. The change in palatability of the controls and of the samples exposed to sunlight (average for samples from both sources) was as follows:

Storage time in days	0	13	23	39	48
Palatability (control)	2.2	3.6	5.0	4.3	5.6
Palatability (exposed)	2.2	3.1	3.6	2.5	2.2

The control samples showed some tendency to increase in palatability throughout the storage period, while the exposed samples increased slightly initially and then decreased.

TABLE I

THE EFFECT OF LIGHT, SOURCE OF POWDER, AND TIME OF EXPOSURE ON
THE QUALITY OF SPRAY-DRIED SKIM MILK

Storage conditions			
Sunlight (av. temp. 6° C.)		Ultra-violet (temp. 38° C.)	
Variable under study	Mean palatability	Variable under study	Mean palatability
Treatment		Treatment	
Expo ed to light	2.8	Exposed to light	3.9
Cont ol	4.6	Control	4.2
Source of powder		Source of powder	
X	4.0	X	4.6
Y	3 4	Y	3.5
Time		Time	
Storage (days), sunlight (hr.)		Storage (days), ultra-violet (hr.)	
0 0	2.2	0 0	2.2
13 60	3.4	3 25	4.7
23 110	4.2	13 75	3.9
39 165	3 4	22 150	3.5
48 215	3.9	42 300	4.0

Analysis of variance

Variance attributable to:	Degrees of freedom	Mean square	
		Sunlight	Ultra-violet light
Treatment	1	12.42*	0.25
Source of powder	1	1.38	5.52**
Time	3	0.75	0.91
Residual	10	1.26	0 52

* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% level of statistical significance.*

Since skim milk of only 1% fat is affected by sunlight, it seems reasonable to assume that sunlight affected not only the fat fraction of the milk powder, but has some effect on the protein or carbohydrate components, or both.

The Effect of Light Intensity on Keeping Quality

The results of storing milk powders of 1% and 26% fat (initial palatabilities 6.9 and 6.3, respectively), under light of different intensities is shown in Table II. While the palatability scores applied at each trial differed significantly, the intensity of light falling on the sample caused significant differences in rate of deterioration. Skim milk powders did not deteriorate markedly except at the greatest light intensity, while whole milk powders were affected even by the light of lowest intensity, lending support to the previous observa-

TABLE II

THE EFFECT OF LIGHT ENERGY ON THE QUALITY OF SPRAY-DRIED WHOLE AND SKIM MILK
POWDER STORED FOR THREE DAYS AT 38° C. (100° F.)

Table of means

Light energy affecting sample, cal./sq. cm./min.	Palatability scores			
	1% fat		26% fat	
	Trial 1	Trial 2	Trial 1	Trial 2
0	5.7	6.5	5.6	5.9
0.00038	5.9	6.6	3.8	5.1
0.0036	4.8	5.5	3.6	4.9

Analysis of variance

Source of variance	D. f.	Mean square
1% fat vs. 26% fat	1	31.01**
Between trials	2	11.04**
Light energy	2	15.02**
Light energy × fat levels	2	20.86**
Residual (Error I)	58	1.56
Tasters	9	3.80**
Residual (Error II)	45	0.64

** *Exceeds 1% level of statistical significance.*

tions (Fig. 1 and Table I). The high initial palatability of the skim milk was attributed to the fact that the container had been opened and was returned to storage for some time before the initiation of the experiment.

Since the light source used here produced a continuous spectrum from 2800 Å units far into the infra red, it was believed that the deterioration in relation to the energy expended might indicate whether sunlight taint of this product was a function of wave length or a function of the total energy. The results of calculations relating palatability decrease to light energy, shown in Fig. 2, seemed to indicate that deterioration was a function of wave length rather than the total energy expended on the sample. However, the palatability decrease of the control sample of whole milk powder subjected to the incandescent light was 2.5 times that occurring in the samples exposed to ultra-violet light. If comparable palatability decreases are assumed for both sets of control samples, the calculated decrease in the sample exposed to ultra-violet light (dotted curve in Fig. 2) almost equals the decrease in palatability occurring at corresponding light energy of the incandescent lamp. These calculations indicated that wave length of the incident light was a less important cause of deterioration than the energy expended on the samples.
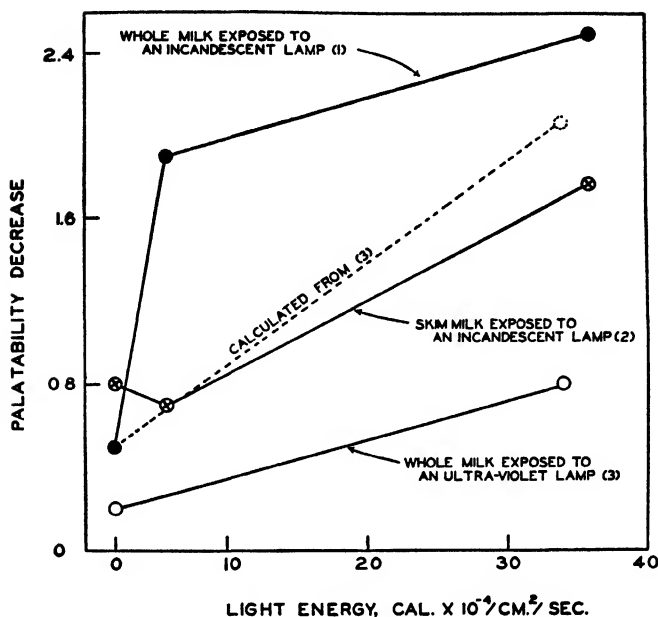
FIG. 2. *The relation between light energy expended on the sample and palatability decrease. Dotted line shows values anticipated if milk powder exposed to the ultra-violet lamp had been as unstable as the powder exposed to the incandescent lamp.*

Acknowledgments

References

1. BRYCE, W. A. and PEARCE, J. A.   Can. J. Research.   In press.
2. DAVIES, W. L.   The chemistry of milk.   Chapman and Hall, Ltd., London.   1936.
3. LEA, C. H.   Rancidity in edible fats.   Dept. Sci. Ind. Research (Brit.) Food Invest. Special Rept. No. 46.   His Majesty's Stationery Office, London.   1938.
4. PEARCE, J. A.   Can. J. Research, F, 22 : 87-95.   1944.
5. PEARCE, J. A.   Can. J. Research, F, 23 : 9-21.   1945.
6. PEARCE, J. A.   Can. J. Research, F, 23 : 177-184.   1945.
7. WOODCOCK, A. H.   Can. J. Research, F, 23 : 117-122.   1945.

CANADIAN WILTSHIRE BACON

XXV. CHEMICAL PRESERVATIVES FOR MAINTAINING QUALITY AT HIGH STORAGE TEMPERATURES

By W. Harold White, N. E. Gibbons, and M. W. Thistle

CANADIAN WILTSHIRE BACON

XXV. CHEMICAL PRESERVATIVES FOR MAINTAINING QUALITY AT HIGH STORAGE TEMPERATURES[1]

By W. Harold White[2], N. E. Gibbons[3], and M. W. Thistle[4]

Abstract

Seventy-seven chemical treatments were tested for their effectiveness in maintaining quality in bacon stored at 7.1°, 15.6°, and 23.8° C. for 30 days.   The relative suitability of the compounds was assessed by organoleptic examination.

Treatments found to retard both slime formation and mould growth include magnesium benzoate with hydroxyacetic or citric acid; dimethylolurea; borobenzoic acid; acetylsalicylic acid; Aerosol-OS; Aseptex; Salol; cinnamic acid; and a mixture of benzoic acid, citric acid, salt, and oat flour and hulls.   Several of the materials were relatively effective against bacteria but not against moulds, viz.: magnesium benzoate; sodium benzoate with hydroxyacetic acid; benzoic or boric acid with hydroxyacetic acid; cheesecloth treated with formaldehyde; and pyruvic acid.   A few of the treatments, e.g. borax and Nacconal, retarded mould growth, but had little effect on bacteria.   Because of possible toxicity or other considerations, none of the materials studied is considered to be entirely satisfactory.

Introduction

Under normal shipping conditions Canadian Wiltshire bacon sometimes arrived in England showing slime and other signs of incipient spoilage.   The problem became more serious in the early war period because of unpredictable delays and the possible need for shipment in unrefrigerated space.   Hence some precautionary measures were necessary to ensure that the bacon reached its destination in an edible and acceptable condition.   Extensive investigations were carried out in these laboratories on the relative efficacy of smoking, of modifications in the curing process, and of chemical preservatives in maintaining the quality of bacon during longer holding periods, or at higher temperatures, than usual.   Studies on smoking have been reported (8, 9,10).   The present paper gives the results of a study on the effectiveness of 77 chemical treatments in retarding spoilage of bacon stored at 7.1°, 15.6°, and 23.8° C.

The status of the use of preservatives in Canada has been ably discussed elsewhere and the literature on their application to fish products reviewed (5).   This review and other recent investigations (1, 2, 3, 4, 7) have dealt mainly with the action of derivatives of benzoic acid.   Since meat is more resistant to spoilage than fish and adequate refrigeration facilities are usually available,

there has been little incentive to search for chemical preservatives for this class of product. A recent review on the preservation of meat confirms this (6).

The British Ministry of Food early realized the necessity of providing some protection for bacon and authorized the use of borax or boric acid on Wiltshire sides*. Since these have relatively little preservative action, a search was made for more effective and less toxic chemicals. Accordingly, the value of most of the well-known preservatives was assessed, and many other compounds were investigated in an attempt to attain this objective. In the study of these hitherto untested compounds, secondary consideration was given to their toxicity since it was considered that ineffective substances could be discarded, while effective compounds, even if toxic, might give an indication of a suitable treatment. It is recognized that thorough toxicological studies would be required before any treatment could be considered for commercial adoption.

Materials and Procedure

Freshly cured Wiltshire backs were divided into five portions, each approximately 3 lb. in weight. The resulting samples were randomized amongst the various treatments and storage temperatures.

The preservatives studied, arranged where possible according to their chemical nature or type of treatment, are listed in Table II. For the most part, each preservative was tested at three concentrations for each of the storage temperatures, 7 1°, 15.6°, and 23.8° C. (45°, 60°, and 75° F.). The amount of preservative added was calculated as the percentage of the lean in an average sized back weighing 15 lb. and having 60% lean meat. On this basis, 0.8 gm. added to the 3-lb. test pieces approximated 0.1% of the lean meat. When the preservative was admixed with hydroxyacetic or citric acid, 0.8 gm. of the acid was thoroughly mixed with the desired weight of preservative. Depending on their physical condition, solid materials were either dusted or sprinkled onto the samples. Unless otherwise indicated, liquids were applied with a brush. When second and third applications of a liquid were made, sufficient time elapsed between successive treatments to permit at least superficial sorption of the preservative to occur. All preservatives were applied to the rib or top surfaces only of the lean meat. Each treated sample was wrapped in waxed, and overwrapped with brown, paper prior to storage.

Subjective examinations of the condition of each sample with respect to the surface colour, odour, slime formation, and mould growth for the lean meat were made after storage for 15 and 30 days.

Results

Assessment of the relative effectiveness of the various treatments from the data in the form of comments was difficult. An arbitrary system of scoring

*S. R. and O. for Foods, 1940, No. 547 (Food, 9 : 236. 1940).

quality was therefore adopted (Table I), based on the following considerations. The development of off-odours in the lean meat was believed to be the most important factor studied, since spoilage of the lean renders the bacon inedible.   Surface growths of either bacteria or moulds detract from the appear-

TABLE I

SYSTEM OF SCORING EMPLOYED FOR ASSESSING THE RELATIVE SUITABILITY
OF PRESERVATIVES FOR WILTSHIRE BACON

Property	Score	Property	Score
Odour of the lean:		Mould:	
Good	3	None or slight	2
Slightly off or chemical	2	Medium	1
Definitely off	1	Heavy	0
Bad	0		
Slime:		Colour of the lean:	
None or slight	2	Acceptable	1
Medium	1	Poor	0
Heavy	0		

ance of the bacon, but, if not too far advanced and not accompanied by off-odours, they are considered to be of secondary importance since either can, for the most part, be removed readily by mechanical methods.   The surface colour of the lean meat, as an attribute of quality, was ranked third in importance.

Quality scores for bacon, treated with the different amounts studied of the various preservatives and stored at 7.1°, 15.6°, and 23.8° C. (45°, 60°, and 75° F.) for 30 days, are given in Table II.   It was considered that the above information gives sufficient indication of the relative effectiveness of the various treatments.   Although presentation of other data, such as the detailed observations of the conditions of the samples, would be desirable, it is impractical because of the large number of treatments studied.

When bacon is stored without preservative for 30 days at 7.1° C., the odour of the lean is usually off and there may be some slime; at 15.6° C., the odour is definitely off and the surface is quite slimy; at 23.8° C., the meat is very bad.

For the most part, all treatments gave relatively good protection to the bacon stored at 7.1° C. Obvious differences were evident, however, at 15.6° C. and 23.8° C.   The addition of citric or hydroxyacetic acid to the preservative usually had a beneficial effect.   The use of hydroxyacetic acid appears to be preferable economically but, owing to its hygroscopic character, has the disadvantage of difficulty of application.   It is also of some interest to note that in certain instances the preservative was more effective at 23.8° than at 15.6° C.   The reason for this is not apparent.

Detailed examination of the total quality scores indicated that the following treatments were the most effective in retarding spoilage: benzoic acid with

TABLE II

QUALITY SCORES FOR BACON TREATED WITH VARIOUS PRESERVATIVES AND STORED
AT 7.1°, 15.6°, AND 23.8° C. FOR 30 DAYS

No.	Preservative		Score[1] at various storage temperatures		
	Treatment	Amount	7.1° C.	15.6° C.	23.8° C.
1	Benzoic acid	0.8 gm.	3220–7	1200–3	3200–5
		1.6 gm.	3220–7	0201–3	1200–3
		4.0 gm.	3221–8	1200–3	2220–6
2	Benzoic acid with citric acid	0.8 gm.	3221–8	1210–4	2210–5
		1.6 gm.	3221–8	2220–6	1210–4
		4.0 gm.	3221–8	3201–6	2220–6
3	Benzoic acid with hydroxyacetic acid	0.8 gm.	3221–8	3211–7	0210–3
		1.6 gm.	3221–8	3210–6	2211–6
		4.0 gm.	3220–7	3220–7	2221–7
4	Benzyl benzoate	Once	3220–7	1021–4	0220–4
		Twice	3220–7	1221–6	0010–1
		Thrice	3220–7	1020–3	0020–2
5	Ethyl benzoate	Once	2220–6	2210–5	1221–6
		Twice	2221–7	3211–7	3110–5
		Thrice	2221–7	3211–7	0101–2
6	Phenyl benzoate	0.8 gm.	2220–6	0220–4	1221–6
		1.6 gm.	2220–6	2220–6	2221–7
		4.0 gm.	2221–7	2221–7	2220–6
7	Methyl ester of *p*-hydroxybenzoic acid	0.4 gm.	2221–7	1210–4	1000–1
		0.8 gm.	2220–6	1220–5	2000–2
		1.6 gm.	2221–7	1220–5	2120–5
8	Ethyl ester of *p*-hydroxybenzoic acid	0.4 gm.	3221–8	0020–2	1100–2
		0.8 gm.	3220–7	2220–6	2220–6
		1.6 gm.	3221–8	2220–6	0220–4
9	Ethyl ester of *p*-hydroxybenzoic acid with citric acid	0.4 gm.	3221–8	3210–6	0220–4
		0.8 gm.	3221–8	2220–6	2200–4
		1.6 gm.	3221–8	2220–6	2200–4
10	Propyl ester of *p*-hydroxybenzoic acid	0.4 gm.	3221–8	1020–3	1010–2
		0.8 gm.	3221–8	1220–5	1120–4
		1.6 gm.	3221–8	0010–1	0020–2
11	Butyl ester of *p*-hydroxybenzoic acid	0.4 gm.	2221–7	0000–0	2220–6
		0.8 gm.	2021–5	0020–2	0020–2
		1.6 gm.	2220–6	0020–2	2220–6
12	Benzyl ester of *p*-hydroxybenzoic acid	0.4 gm.	2220–6	0021–3	0000–0
		0.8 gm.	3220–7	0121–4	0120–3
		1.6 gm.	3221–8	0120–3	0020–2
13	Sodium salt of methyl ester of *p*-hydroxybenzoic acid	0.4 gm.	2220–6	2220–6	0120–3
		0.8 gm.	2220–6	2220–6	0020–2
		1.6 gm.	2220–6	2220–6	3220–7

[1] *The first digit indicates the rating assigned for the odour of the lean meat; the second and third for slime and mould, respectively; the fourth for the colour of the lean meat; and the fifth, the total score. Further details of the method of scoring are given in Table I.*

TABLE II—*Continued*

QUALITY SCORES FOR BACON TREATED WITH VARIOUS PRESERVATIVES AND STORED
AT 7.1°, 15.6°, AND 23.8° C. FOR 30 DAYS—*Continued*

No.	Preservative		Score[1] at various storage temperatures		
	Treatment	Amount	7.1° C.	15.6° C.	23.8° C.
14	Sodium salt of ethyl ester of *p*-hydroxybenzoic acid	0.4 gm.	3220–7	1220–5	0120–3
		0.8 gm.	2220–6	2200–4	2200–4
		1.6 gm.	2220–6	1220–5	0220–4
15	Sodium salt of propyl ester of *p*-hydroxybenzoic acid	0.4 gm.	3220–7	2120–5	0100–1
		0.8 gm.	3220–7	1220–5	1200–3
		1.6 gm.	3221–8	1200–3	3220–7
16	Sodium benzoate	0.8 gm.	2120–5	1101–3	0010–1
		1.6 gm.	3221–8	0101–2	0010–1
		4.0 gm.	3220–7	1211–5	1220–5
17	Sodium benzoate with citric acid	0.8 gm.	3221–8	1200–3	0000–0
		1.6 gm.	3221–8	2101–4	1100–2
		4.0 gm.	3221–8	2100–3	1100–2
18	Sodium benzoate with hydroxy-acetic acid	0.8 gm.	3221–8	0221–5	3220–7
		1.6 gm.	3221–8	2220–6	3220–7
		4.0 gm.	3221–8	3220–7	0220–4
19	Magnesium benzoate	0.8 gm.	3221–8	0010–1	0100–1
		1.6 gm.	3221–8	0210–3	1210–4
		4.0 gm.	3221–8	3221–8	3220–7
20	Magnesium benzoate with citric acid	0.8 gm.	3220–7	3220–7	0110–2
		1.6 gm.	3220–7	2220–6	0100–1
		4.0 gm.	3221–8	3220–7	3220–7
21	Magnesium benzoate with hydroxyacetic acid	0.8 gm.	3221–8	0221–5	0010–1
		1.6 gm.	3221–8	3220–7	3200–5
		4.0 gm.	3220–7	3220–7	1210–4
22	Acetylsalicylic acid	1.6 gm.	2221–7	2221–7	2200–4
		4.0 gm.	3221–8	3220–7	2200–4
		8.0 gm.	3221–8	3220–7	3220–7
23	Phthalic acid with citric acid	0.8 gm.	3221–8	1200–3	0200–2
		1.6 gm.	3210–6	0201–3	0100–1
		4.0 gm.	1120–4	0221–5	1200–3
24	Sulphanilic acid with citric acid	0.8 gm.	3210–6	1100–2	0200–2
		1.6 gm.	3210–6	2200–4	0000–0
		4.0 gm.	3210–6	3210–6	0200–2
25	Dupont's No. IN-3102; 2% solution in petroleum ether	Once	3221–8	0021–3	0220–4
		Twice	2121–6	0020–2	0021–3
		Thrice	2220–6	0120–3	0020–2
26	Cinnamic acid with citric acid	0.8 gm.	3221–8	3220–7	3220–7
		1.6 gm.	3221–8	2220–6	3220–7
		4.0 gm.	3221–8	2220–6	2220–6

[1] *The first digit indicates the rating assigned for the odour of the lean meat; the second and third for slime and mould, respectively; the fourth for the colour of the lean meat; and the fifth, the total score. Further details of the method of scoring are given in Table I.*

TABLE II—*Continued*

QUALITY SCORES FOR BACON TREATED WITH VARIOUS PRESERVATIVES AND STORED
AT 7.1°, 15.6°, AND 23.8° C. FOR 30 DAYS—*Continued*

No.	Preservative		Score[1] at various storage temperatures		
	Treatment	Amount	7.1° C.	15.6° C.	23.8° C.
27	Sodium sulphocarbolate	0.8 gm.	2221–7	0021–3	0221–5
		1.6 gm.	3221–8	0221–5	0120–3
		4.0 gm.	3221–8	0020–2	0021–3
28	Borobenzoic acid with citric acid	0.8 gm.	3221–8	2210–5	3200–5
		1.6 gm.	3221–8	3220–7	3220–7
		4.0 gm.	3220–7	3220–7	3220–7
29	Boric acid	1.6 gm.	3221–8	0100–1	2200–4
		4.0 gm.	3220–7	1201–4	2200–4
		8.0 gm.	3220–7	1201–4	2210–5
30	Boric acid with citric acid	1.6 gm.	3221–8	2211–6	1100–2
		4.0 gm.	3221–8	2201–5	1200–3
		8.0 gm.	3221–8	2221–7	1201–4
31	Boric acid with hydroxyacetic acid	0.8 gm.	3220–7	1210–4	3220–7
		1.6 gm.	3220–7	3220–7	0210–3
		4.0 gm.	3221–8	3221–8	2210–5
32	Boric anhydride with citric acid	0.8 gm.	3221–8	3210–6	2210–5
		1.6 gm.	3221–8	3211–7	1221–6
		4.0 gm.	3221–8	3221–8	0221–5
33	Borax	1.6 gm.	2220–6	1021–4	0021–3
		4.0 gm.	3220–7	0021–3	1121–5
		8.0 gm.	3220–7	0021–3	1121–5
34	Borax with citric acid	1.6 gm.	2220–6	1220–5	0110–2
		4.0 gm.	3220–7	2220–6	2220–6
		8.0 gm.	3220–7	1221–6	2221–7
35	Ethyl alcohol (95%); dipped 15 min.	—	2220–6	0200–2	3000–3
36	Ethyl alcohol (95%) with 2% hydroxyacetic acid; pH 3.35	Once	3200–5	0001–1	0220–4
		Twice	3210–6	0111–3	0001–1
		Thrice	3201–6	1010–2	0000–0
37	Ethylene glycol	Once	1021–4	0021–3	0001–1
		Twice	3020–5	0020–2	0101–2
		Thrice	1020–3	0020–2	0020–2
38	Propylene glycol	Once	2221–7	0020–2	0120–3
		Twice	3121–7	0021–3	0021–3
		Thrice	3121–7	0021–3	0020–2
39	Glycerol with hydroxyacetic acid; 50 gm. acid dissolved in 200 ml. water and added to 800 ml. glycerine; pH 1.8	Once	3220–7	3201–6	0101–2
		Twice	3111–6	3201–6	0001–1
		Thrice	3221–8	3200–5	3210–6

[1] *The first digit indicates the rating assigned for the odour of the lean meat; the second and third for slime and mould, respectively; the fourth for the colour of the lean meat; and the fifth, the total score. Further details of the method of scoring are given in Table I.*

TABLE II—*Continued*

No.	Preservative		Score[1] at various storage temperatures		
	Treatment	Amount	7.1° C.	15.6° C.	23.8° C.
40	Anhydrous sodium sulphate	4.0 gm.	1020–3	0221–5	0220–4
		8.0 gm.	3220–7	0021–3	0020–2
		16.0 gm.	3221–8	0220–4	0221–5
41	Anhydrous calcium chloride	4.0 gm.	3221–8	0020–2	1120–4
		8.0 gm.	3221–8	0010–1	0020–2
		16.0 gm.	3221–8	0001–1	0000–0
42	Oil of birch	Once	2220–6	2220–6	2220–6
		Twice	2220–6	2220–6	3200–5
		Thrice	2121–6	2220–6	2000–2
43	Oil of birch tar	Once	3120–6	0020–2	2020–4
		Twice	2120–5	2010–3	2020–4
		Thrice	2220–6	2021–5	2020–4
44	Oil of pine	Once	2221–7	2120–5	2120–5
		Twice	2220–6	2220–6	2220–6
		Thrice	2221–7	2121–6	2020–4
45	Oil of tar	Once	2220–6	2110–4	3210–6
		Twice	2220–6	2210–5	2210–5
		Thrice	2220–6	2220–6	2110–4
46	Essence of smoke	Once	3210–6	3210–6	3210–6
		Twice	3220–7	2100–3	3200–5
		Thrice	3220–7	2110–4	3210–6
47	Sawdust	Excess	3221–8	0221–5	3221–8
48	Wiping with rag treated with 1% formaldehyde solution	—	3021–6	0020–2	0000–0
49	Wiping with rag treated with glacial acetic acid	—	3221–8	2201–5	3201–6
50	Wiping with rag treated with saturated solution of benzoic acid containing 2% hydroxyacetic acid	—	2220–6	0220–4	0000–0
51	Dipping in aqueous solution of formaldehyde for one minute	2%	3220–7	3201–6	1100–2
		5%	3220–7	0110–2	0110–2
52	Wrapping in cheesecloth treated with formaldehyde	1%	3221–8	0111–3	0001–1
		3%	3220–7	0111–3	3211–7
		5%	3221–8	0020–2	3201–6
53	Sodium propionate	1.6 gm.	3221–8	2210–5	0010–1
		4.0 gm.	2221–7	2011–4	0020–2
		8.0 gm.	2221–7	2210–5	1110–3

[1] *The first digit indicates the rating assigned for the odour of the lean meat; the second and third for slime and mould, respectively; the fourth for the colour of the lean meat; and the fifth, the total score. Further details of the method of scoring are given in Table I.*

TABLE II—*Continued*

QUALITY SCORES FOR BACON TREATED WITH VARIOUS PRESERVATIVES AND STORED
AT 7.1°, 15.6°, AND 23.8° C. FOR 30 DAYS—*Continued*

No.	Preservative		Score[1] at various storage temperatures		
	Treatment	Amount	7.1° C.	15.6° C.	23.8° C.
54	Sodium propionate with citric acid	1.6 gm.	3220–7	2200–4	1101–3
		4.0 gm.	2220–6	1200–3	2210–5
		8.0 gm.	2221–7	2221–7	1101–3
55	Urotropin	1.6 gm.	2220–6	0000–0	1100–2
		4.0 gm.	3111–6	2201–5	2100–3
		8.0 gm.	3220–7	2200–4	2200–4
56	Nickel pectinate (flake)	0.8 gm.	2221–7	0220–4	0220–4
		1.6 gm.	3220–7	0220–4	0220–4
		4.0 gm.	1220–5	0020–2	0120–3
57	Sodium pyrophosphate with hydroxyacetic acid	0.8 gm.	3220–7	1221–6	0201–3
		1.6 gm.	3121–7	0220–4	0221–5
		4.0 gm.	3221–8	0220–4	0221–5
58	Sulphamic acid with citric acid	0.8 gm.	3221–8	1201–4	1220–5
		1.6 gm.	3211–7	3200–5	0200–2
		4.0 gm.	0220–4	3220–7	1200–3
59	Pyruvic acid	Once	3220–7	3211–7	3200–5
		Twice	3220–7	3210–6	3211–7
		Thrice	3221–8	3220–7	3201–6
60	Hydroxyacetic acid	0.8 gm.	3221–8	0220–4	0010–1
		1.6 gm.	3221–8	0121–4	0000–0
		4.0 gm.	3221–8	0101–2	3200–5
61	Citric acid	0.8 gm.	3200–5	0100–1	0001–1
		1.6 gm.	3121–7	1000–1	2201–5
		4.0 gm.	3210–6	1100–2	0100–1
62	Hydrochloric acid; dipped 15 min.	0.05 *N*	3210–6	2200–4	0000–0
		0.1 *N*	2220–6	0200–2	0000–0
		0.5 *N*	2201–5	1200–3	2100–3
63	Zephirin; dipped 15 min.	1 : 5000	2011–4	0010–1	0000–0
		1 : 10, 000	2120–5	0011–2	0000–0
		1 : 20, 000	2220–6	0000–0	0100–1
64	Moldex	0.8 gm.	3221–8	1220–5	2220–6
		1.6 gm.	3220–7	2221–7	2220–6
		4.0 gm.	2220–6	2220–6	2220–6
65	Aseptex	0.8 gm.	3220–7	2111–5	0210–3
		1.6 gm.	3220–7	3201–6	0200–2
		4.0 gm.	3120–6	3220–7	3220–7
66	Salol	0.8 gm.	3220–7	0020–2	2220–6
		1.6 gm.	2221–7	2220–6	1121–5
		4.0 gm.	2220–6	2221–7	2220–6

[1] *The first digit indicates the rating assigned for the odour of the lean meat; the second and third for slime and mould, respectively; the fourth for the colour of the lean meat; and the fifth, the total score. Further details of the method of scoring are given in Table I.*

TABLE II—*Concluded*

QUALITY SCORES FOR BACON TREATED WITH VARIOUS PRESERVATIVES AND STORED
AT 7.1°, 15.6°, AND 23.8° C. FOR 30 DAYS—*Concluded*

No.	Preservative		Score[1] at various storage temperatures		
	Treatment	Amount	7.1° C.	15.6° C.	23.8° C.
67	Aerosol-OS	0.8 gm.	3221–8	1121–5	1221–6
		1.6 gm.	3221–8	0021–3	3221–8
		4.0 gm.	3221–8	3221–8	3221–8
68	Nacconal	0.8 gm.	3220–7	2221–7	0120–3
		1.6 gm.	3220–7	3221–8	0020–2
		4.0 gm.	3221–8	3220–7	2220–6
69	Allantoin	0.8 gm.	3221–8	0220–4	0021–3
		1.6 gm.	3221–8	0021–3	0220–4
		4.0 gm.	3220–7	0021–3	0021–3
70	Dimethylol urea	1.6 gm.	3220–7	3211–7	3220–7
		3.2 gm.	3220–7	3220–7	3210–6
		8.0 gm.	3220–7	3220–7	3220–7
71	Sodium hydroxide (flake)	1.0 gm.	3220–7	0221–5	0220–4
		4.0 gm.	3220–7	3020–5	3220–7
		7.0 gm.	3220–7	3020–5	3220–7
		10.0 gm.	3220–7	3020–5	3220–7
72	Oat hulls	Excess	3220–7	0221–5	0221–5
73	Sodium bicarbonate and citric acid; packed in oat hulls; NaHCO₃: citric acid = 252 : 192	10 gm.	3221–8	0221–5	0201–3
		20 gm.	3221–8	2221–7	0221–5
		30 gm.	3220–7	0221–5	0221–5
74	Sodium bicarbonate and hydroxy-acetic acid; packed in oat hulls; NaHCO₃ : CH₂OH.COOH = 84 : 76	10 gm.	3221–8	0221–5	0210–3
		20 gm.	3221–8	2221–7	0211–4
		30 gm.	3221–8	2221–7	0210–3
75	Benzoic acid (4 parts), citric acid (2 parts), sodium chloride (20 parts), oat flour (46 parts), and oat hulls (28 parts)	Excess	3221–8	3221–8	3221–8
76	Surface of meat dried and scorched by a gas torch	—	3220–7	0000–0	2110–4
77	Sodium pyrophosphate	0.8 gm.	3221–8	3221–8	0020–2
		1.6 gm.	3221–8	0020–2	0021–3
		4.0 gm.	3221–8	0220–4	0021–3

[1] *The first digit indicates the rating assigned for the odour of the lean meat; the second and third for slime and mould, respectively; the fourth for the colour of the lean meat; and the fifth, the total score. Further details of the method of scoring are given in Table I.*

hydroxyacetic acid; phenyl benzoate; sodium benzoate with hydroxyacetic acid; magnesium benzoate with and without hydroxyacetic acid; acetyl-salicylic acid; cinnamic acid with citric acid; borobenzoic acid with citric acid; boric acid with hydroxyacetic acid; glycerol with hydroxyacetic acid;

packing in sawdust; wrapping in cheesecloth treated with formaldehyde; pyruvic acid; Aseptex; Salol; Aerosol-OS; dimethylolurea; sodium hydroxide; and a mixture of benzoic acid, citric acid, sodium chloride, and oat flour and hulls.

A group of treatments of an intermediate degree of effectiveness included: benzoic acid with citric acid; benzyl and ethyl benzoates; ethyl ester of *p*-hydroxybenzoic acid with citric acid; propyl and benzyl esters of *p*-hydroxybenzoic acid; sodium salts of methyl, ethyl, and propyl esters of *p*-hydroxybenzoic acid; magnesium benzoate with citric acid; boric acid with and without citric acid; boric anhydride with citric acid; borax with and without citric acid; dipping for 15 min. in ethyl alcohol; oils of birch, birch tar, pine, and tar, essence of smoke; wiping with rags treated with glacial acetic acid; dipping for one minute in an aqueous solution of formaldehyde; sodium propionate with and without citric acid; urotropin; Moldex; and Nacconal.

Several treatments were found to be quite unsuitable for bacon, viz.: benzoic acid; methyl, ethyl, and butyl esters of *p*-hydroxybenzoic acid; sodium benzoate with and without citric acid; phthalic or sulphanilic acid with citric acid; 2% solution of Dupont's No. IN-3102 in petroleum ether; sodium sulphocarbolate; 95% ethyl alcohol with 2% hydroxyacetic acid; ethylene or propylene glycol; anhydrous sodium sulphate or calcium chloride; wiping with rags treated with 1% formaldehyde solution or with a saturated solution of benzoic acid containing 2% hydroxyacetic acid; nickel pectinate; sodium pyrophosphate with and without hydroxyacetic acid; sulphamic acid with citric acid; hydroxyacetic or citric acids; dipping for 15 min. in hydrochloric acid or in Zephirin; allantoin; oat hulls; dusting with a mixture of sodium bicarbonate with citric or hydroxyacetic acid and then packing in oat hulls; drying and scorching the surface of the meat with a gas torch.

Examination of the data on slime and mould formation indicates that very few of the treatments were effective at the lowest concentration studied. At the highest concentration several treatments were quite effective in retarding both slime formation and mould growth, namely, magnesium benzoate with hydroxyacetic or citric acid; borobenzoic acid; acetylsalicylic acid; Aerosol; Aseptex; Salol; cinnamic acid; dimethylolurea; and a mixture of benzoic acid, citric acid, salt, and oat flour and hulls. Several of the materials were relatively effective against bacteria but not against moulds, viz.: sodium benzoate, phthalic acid or boric acid with citric acid; glycerol with hydroxyacetic acid; essence of smoke; and pyruvic acid. A few of the treatments retarded mould growth, but had little effect on bacteria, e.g. borax, ethylene or propylene glycol, and benzyl benzoate.

Discussion

The "ideal" preservative for bacon would be one that is non-toxic, even if ingested repeatedly, and that would prevent the growth of bacteria and moulds and the development of rancidity. It should have no effect on the colour, flavour, odour, or texture of the meat. The method of application

should fit into normal plant practice, and the cost should be as reasonable as possible. None of the preservatives studied here can be considered to satisfy all of these criteria. Investigations on these and other treatments are being continued.

Acknowledgments

The authors wish to express their appreciation to Mr. T. A. Steeves, formerly Refrigeration Engineer, National Research Laboratories, for establishing and maintaining the storage conditions, and to L. Moore and R. Moore, Laboratory Assistants, for their assistance with the investigations.

References

1. ANON. Food Manuf. 9 : 102-104. 1934.
2. BEHRE, A. Chem. Ztg. 54 : 325-327; 346-347. 1930.
3. CROWTHER, E. H. Can. Chem. Process Inds. 24 : 505; 521. 1940.
4. FELLERS, C. R. and HARVEY, E. W. Food Research, 5 : 1-12. 1940.
5. HESS, E. Can. Chem. Process Inds. 23 : 495-499. 1939.
6. JONES, O. Food, 11 : 25-27. 1942.
7. TARR, H. L. A. and SUNDERLAND, P. A. J. Fisheries Research Board Canada, 5 : 148-163. 1940.
8. WHITE, W. H. Can. J. Research, F, 22 : 97-106. 1944.
9. WHITE, W. H., GIBBONS, N. E., WOODCOCK, A. H., and COOK, W. H. Can. J. Research, D, 20 : 263-275. 1942.
10. WHITE, W. H., WOODCOCK, A. H., and GIBBONS, N. E. Can. J. Research, F, 22 : 107-118. 1944.

CANADIAN WILTSHIRE BACON

XXVI. FURTHER OBSERVATIONS ON THE PRESERVATION OF QUALITY AT HIGH STORAGE TEMPERATURES[1]

BY W. HAROLD WHITE[2], M. W. THISTLE[3], AND T. A. STEEVES[4]

Abstract

The suitability of polyvinyl alcohols, dimethylol urea, commercial wax dispersions, carbon dioxide, sodium hydroxide, and a combined smoking and partial desiccation treatment as preservatives for bacon stored at 15 6° C. (60° F.) was investigated. Dimethylol urea was a relatively effective preservative and mouldicide for smoked bacon. Of the methods studied for applying this material, a vaseline paste was most suitable, but not entirely satisfactory. Back bacon, smoked for six hours at 40.6° C. (105° F.), and subsequently dried at this temperature to moisture contents of about 55 to 60% or less, was in a satisfactory condition, except for a heavy mould growth, after 60 days' storage. Sodium hydroxide was relatively ineffective when applied as an aqueous solution. Polyvinyl alcohols, wax dispersions, and carbon dioxide were unsuitable under the conditions studied.

Introduction

Extensive investigations have been made in these laboratories on methods for the prevention of spoilage of bacon during storage at high temperatures, of which the effectiveness of smoking (4, 8, 9), strong curing (6), and a number of chemical treatments (7) have been described previously. The present paper deals with further observations made on chemical preservatives. In addition, the effect of partial desiccation on the keeping quality of bacon is described.

Polyvinyl Alcohols

The properties of the polyvinyl alcohols would appear to make these materials worthy of investigation as protective coverings for food products. Being water-soluble, they may be directly applied by immersion of the food in an aqueous solution of the alcohol, thus avoiding the use of volatile solvents normally required for most synthetic plastics, and the consequent possible tainting of the foodstuff. Moreover, it has been stated that polyvinyl alcohol films are resistant to both moulds and bacteria (1, p. 2). Their use in the preservation of shell eggs has been described (2).

A preliminary experiment was made to ascertain the relative suitability of Types *A* and *B* polyvinyl alcohols (1), the concentration required to give a satisfactory film, and the effect of varying the immersion time of bacon in the polyvinyl alcohol. For this purpose, two thin slices of Wiltshire back bacon

were dipped for each of one and three minutes in the following solutions of polyvinyl alcohol*: Type *A*, low (No. 623) and medium viscosity (No. 488), 2, 6, 10, 14, and 18%; Type *B*, medium viscosity (No. 349), 2, 6, 10, 14, and 18%; and Type *B*, high viscosity (No. 391), 2, 6, and 10%. (All concentrations are expressed as gm. of polyvinyl alcohol per 100 ml. of water.) After dipping, the slices were permitted to drain and dry at laboratory temperature.

Visual examination indicated that the Type *B* polyvinyl alcohols gave much superior films to those of Type *A* at all concentrations, and that the most desirable concentration of the Type *B* polyvinyl alcohols was 14% for the medium viscosity (No. 349) and 10% for the high viscosity (No. 391) polymers. There was no evident difference between samples dipped for one and three minutes.

With this preliminary information available a study was made of the effectiveness of Type *B* polyvinyl alcohol as a preservative for Wiltshire bacon. Two backs, removed from the right and left sides of the same hog, were each cut into nine pieces. Two pieces were allocated at random to each of the following treatments with medium viscosity (No. 349) polyvinyl alcohol: (1) 7% aqueous solution; (2) 10% aqueous solution; (3) 14% aqueous solution; (4) 7% aqueous solution; hydrogen ion concentration adjusted acid to methyl red; (5) 14% aqueous solution; hydrogen ion concentration adjusted acid to methyl red; (6) 7% aqueous solution containing 0.2 parts dimethylol urea to one part of polyvinyl alcohol and hydrogen ion concentration adjusted acid to methyl red; (7) as for (6), but 10% aqueous solution of polyvinyl alcohol; (8) as for (6), but 14% aqueous solution of polyvinyl alcohol; and (9) control.

The small cuts of bacon were dipped in the appropriate solution at room temperature for one minute, drained, and dried in a rapid current of air for approximately one hour. This procedure was repeated, thus giving each sample a double dip. All samples were left for 20 hr. at room temperature to permit drying of the polyvinyl alcohol, wrapped in waxed paper and overwrapped in brown, and stored at 15.6° C. (60° F.) for a period of one month.

The experiment was repeated with the high viscosity (No. 391) polyvinyl alcohol as described above except that concentrations of 6, 9, and 12% were used.

Although it had been planned originally to make detailed chemical and bacteriological examinations of the samples at the end of the storage period, it was found that their condition did not merit more than visual inspection. All samples were putrid, slimy, and mouldy. However, the condition of the treated samples appeared to be slightly better than that of the controls. Medium viscosity polyvinyl alcohol appeared to give slightly more protection than the high viscosity. The addition of acid or dimethylol urea to the polyvinyl alcohol solution had no apparent effect. It may be concluded that the polyvinyl alcohol treatments employed here were unsatisfactory for the preservation of bacon at high storage temperatures.

* The selection of polyvinyl alcohols studied was based on recommendations of Canadian Industries Limited, Montreal.

Dimethylol Urea

The results of previous investigations (4, 8) and of trial shipments to England (5) showed that smoking caused an accelerated growth of moulds on bacon but gave marked protection from other forms of spoilage. In a previous study on chemical preservatives (7), it was observed that dimethylol urea not only retarded putrefaction in unsmoked bacon stored at high temperatures, but prevented mould growth. Accordingly, further studies were made on the application of dimethylol urea to smoked bacon.

Use of Dimethylol Urea on Smoked Bacon

The effect of dimethylol urea on mould growth on smoked bacon was determined for a freshly cured Wiltshire back that had been smoked at an air temperature of 62.8° C. (145° F.) to an internal meat temperature of 51.7° C. (125° F.), and subsequently divided into eight approximately equal portions. Two pieces were allocated at random to each of the following: treatment with 2, 5, and 10 gm. of dimethylol area dusted on the rib and meat surfaces and no treatment (control samples).

After treatment each sample was wrapped in waxed, and overwrapped with brown, paper and stored at 15.6° C. (60° F.) for one month. An organoleptic examination of the samples was made at the end of the storage period.

The results of the examination of the samples are given in Table I. The general condition of the meat treated with the greater amounts of dimethylol

TABLE I

RESULTS OF ORGANOLEPTIC EXAMINATION OF SMOKED BACON TREATED WITH DIMETHYLOL UREA AND STORED AT 15.6° C. FOR ONE MONTH

Wt. of dimethylol urea, gm.	Sample No.	Lean				Fat	
		Colour	Odour	Slime	Mould	Colour	Odour
2	1	Good	Good	None	Heavy	Good	Good
	2	Good	Good	None	Heavy	Good	Good
5	3	Good	Excellent	None	None	Good	Good
	4	Good	Excellent	None	Heavy	Good	Good
10	5	Good	Excellent	None	None	Good	Good
	6	Good	Excellent	None	Slight	Good	Good
Control	7	Good	Fair	None	Heavy	Good	Good
	8	Good	Fair	None	Heavy	Good	Good

urea was excellent. There was every indication that, when the surface was adequately covered, dimethylol urea prevented the growth of moulds on smoked bacon. The somewhat variable results obtained with the use of 5 and 10 gm. of dimethylol urea are attributed to unequal distribution of the material on the surface.

Methods of Application of Dimethylol Urea to Smoked Bacon

In the previous experiment it was shown that dimethylol urea retarded both bacterial and mould growth on bacon if the surface was adequately covered with the material. The object of the present experiment was to determine the most suitable of several possible methods for applying dimethylol urea to bacon.

One freshly smoked Wiltshire back was divided into 10 approximately equal portions. Two pieces were allocated at random to each of five treatments: dimethylol urea as (1) a water paste; (2) a water dip; (3) a water spray; (4) a mineral oil dispersion; and (5) a vaseline paste. The various amounts of chemical added are noted in Table II. Dipped or sprayed samples were allowed to drain for 15 min. Pastes were applied manually. After treatment, the samples were wrapped in waxed paper and overwrapped in brown. Organoleptic examination of the condition of the bacon was made after 30, 45, 60, and 78 days' storage at 15.6° C.

TABLE II

QUANTITIES OF DIMETHYLOL UREA APPLIED BY VARIOUS METHODS TO SMOKED BACON

Treatment medium	Sample No.	Wt. of sample, gm.	Wt. of added material, gm.	Dimethylol urea in added material, %	Dimethylol urea added, gm.
Water paste	1	556	101	55	56
	2	440	87	55	48
Water dip	3	467	59	45	27
	4	458	65	45	29
Water spray	5	445	26	45	12
	6	469	23	45	10
Mineral oil	7	595	171	42	72
	8	465	111	42	47
Vaseline paste	9	501	160	35	56
	10	455	166	35	58

The results obtained for 30 and 78 days' storage are given in Table III. While large amounts of dimethylol urea in a water paste prevented mould development for 78 days, abnormal desiccation of the meat was observed even after 30 days' storage. The water dip and spray treatments were the least effective of those studied. The mineral oil paste was unsuitable because of excessive oiliness of the bacon and packaging materials. The samples receiving the vaseline paste treatment retained a fairly good external appearance throughout storage. No slime was evident on any of the samples. The internal condition of the meat treated with the vaseline paste was markedly superior to that of all other samples (Table IV). It would appear that the addition of dimethylol urea in a vaseline paste was preferable to the other methods of application studied in retaining the over-all quality of bacon. However, none of the treatments is considered to be entirely satisfactory.

TABLE III

RESULTS OF ORGANOLEPTIC EXAMINATION OF SMOKED WILTSHIRE BACON STORED AT 15.6° C.
AND TREATED WITH DIMETHYLOL UREA BY VARIOUS METHODS

| Treatment medium | Days in storage | Sample No. | Lean | | | | Fat | | Comments |
			Colour	Odour	Slime	Mould	Colour	Odour	
Water paste	30	1	Good	Good	None	None	Good	Good	Fairly dry
		2	Sl. dark	Sl. chem.	None	None	Good	Good	Fairly dry
	78	1	Dark; greyed	Sl. chem.	None	None	Good	Good	Very dry
		2	Dark; greyed	Sl. chem.	None	None	Good	Good	Very dry
Water dip	30	3	Sl. dark	Sl. chem.	None	One colony	Good	Good	Dry
		4	Sl. dark	Sl. chem.	None	None	Good	Good	Dry
	78	3	Dark; greyed	Sl. chem.	None	Heavy	Good	Mouldy	Very dry
		4	Dark; greyed	Sl. chem.	None	Heavy	Good	Mouldy	Very dry
Water spray	30	5	Sl. dark	Sl. chem.	None	None	Good	Good	Dry
		6	Sl. dark	Sl. chem.	None	None	Good	Good	Dry
	78	5	Dark; greyed	Sl. chem.	None	Heavy	Good	Mouldy	Very dry
		6	Dark; greyed	Sl. chem.	None	Heavy	Good	Mouldy	Very dry
Mineral oil	30	7	Sl. dark	Good	None	None	Good	Good	Oily
		8	Sl. dark	Good	None	None	Good	Good	Oily
	78	7	Dark; greyed	Sl. chem.	None	None	Sl. dark	Good	Oily
		8	Dark; greyed	Sl. chem.	None	Few	Sl. dark	Good	Oily
Vaseline paste	30	9	Good	Good	None	Very slight	Good	Good	Moist
		10	Good	Good	None	None	Good	Good	Moist
	78	9	Sl. brown*	Sl. chem.	None	Slight	Good	Good	Slightly dry
		10	Sl. brown*	Sl. chem.	None	Slight	Good	Good	Slightly dry

Darkening of the meat indicating slight methaemoglobin formation.

TABLE IV

RESULTS OF ORGANOLEPTIC EXAMINATION OF CUT SURFACE OF SMOKED WILTSHIRE BACON
AFTER TREATMENT WITH DIMETHYLOL UREA, AND STORAGE FOR 78 DAYS AT 15.6° C.

| Treatment medium | Sample No. | Cut meat surface | | | Remarks |
		Colour	Odour	Moisture	
Water paste	1	Dull, unattractive	Chemical	Very dry	Mould infiltration
	2	Dull, unattractive	Chemical	Very dry	Mould infiltration
Water dip	3	Dull, poor	Chemical	Very dry	Mould infiltration
	4	Dull, poor	Chemical	Very dry	Mould infiltration
Water spray	5	Dull, poor	Chemical	Very dry	Interior very dry
	6	Dull, poor	Chemical	Very dry	Interior very dry
Mineral oil	7	Good	Sl. chemical	Sl. dry	Appearance, too oily and unattractive.
	8	Good	Sl. chemical	Sl. dry	Appearance, too oily and unattractive
Vaseline paste	9	Excellent	Very good	Excellent	Interior very well preserved and attractive
	10	Excellent	Very good	Excellent	Appearance, very well preserved and attractive

Wax Dispersions and Dimethylol Urea as Preservatives for Bacon

Two wax dispersions*, referred to as *A* and *B*, were investigated with respect to their preservative effect on bacon when used both alone and in conjunction with dimethylol urea. For this purpose an unsmoked, Wiltshire-cured back was divided into 10 approximately equal portions. Two pieces were allocated at random to each of five treatments: (1) dimethylol urea; (2) Wax Dispersion *A*; (3) Wax Dispersion *B*; (4) dimethylol urea followed by *A*; and (5) dimethylol urea followed by *B*. The dimethylol urea was applied by dipping the bacon in a 45% aqueous slurry, and the wax dispersions, with a spray gun. The samples were drained for 15 min., then wrapped in waxed paper, and overwrapped in brown. Organoleptic examination of the condition of the bacon was made after 15 and 31 days' storage at 15.6° C.

The results are given in Table V. Both wax dispersions failed to protect the meat from spoilage and mould growth. Moreover, the fat developed off-odours and became discoloured. In marked contrast, dimethylol urea gave excellent protection from both bacterial and mould growth. The addition of wax dispersions to dimethylol urea had no apparent supplemental effect.

Carbon Dioxide

It was considered that spoilage of bacon stored at high temperatures might be retarded if treated with a suitable chemical mixture to effect a slow evolution of carbon dioxide gas. In an initial experiment a mixture of 25 gm. of citric acid and 33 gm. of sodium bicarbonate was dusted on the rib and meat surfaces of a piece of back bacon about 4 in. in length. The treated sample was stored in a desiccator at room temperature. It was observed that rather vigorous reaction occurred, and little or no preservative effect was obtained. An attempt was made to retard the reaction by covering the meat surface with about 10 gm. of wood sawdust prior to the addition of the acid–bicarbonate mixture. A measure of preservative action was obtained, but not sufficient to be of practical value.

In a second experiment bacon was stored in latex rubber bags containing carbon dioxide. For this purpose a Wiltshire-cured back was divided into 10 equal portions, each of about 675 gm. in weight. Two pieces were allocated at random to each of the following treatments with solid carbon dioxide: (1) 20 gm.; (2) 15 gm.; (3) 10 gm.; (4) 5 gm.; and (5) control. The appropriate sample was placed in an expanded latex bag, the desired amount of solid carbon dioxide added, and the bag evacuated and sealed. The samples were inspected after 15 and 30 days' storage at 15.6° C.

The results for the organoleptic examination are given in Table VI. None of the concentrations of carbon dioxide employed prevented the spoilage of bacon. The presence of carbon dioxide not only had no apparent inhibitive effect on slime formation, but stimulated the growth of moulds. This latter

* *Du Pont Chemicals No. A-1199 and A-1200, respectively. These were kindly supplied by Canadian Industries Limited, Montreal.*

TABLE V

RESULTS OF ORGANOLEPTIC EXAMINATION OF WILTSHIRE BACON TREATED WITH DIMETHYLOL UREA AND WAX DISPERSIONS, AND STORED AT 15.6° C.

Treatment	Days in storage	Sample No.	Lean				Fat	
			Colour	Odour	Slime	Mould	Colour	Odour
Dimethylol urea	15	1	Sl. light	Sl. chem.	Very moist	None	Satisfactory	Satisfactory
		2	Sl. light	Sl. chem.	Very moist	None	Satisfactory	Satisfactory
	31	1	Sl. brown*	Sl. chem.	None	None	Satisfactory	Satisfactory
		2	Sl. brown*	Sl. chem.	None	None	Satisfactory	Satisfactory
Wax Dispersion A	15	3	Good	Sl. off	None	Medium	Yellow	—
		4	Too red	Bad	Slight	Medium	—	—
	31	3	Too bright; mottled	Bad	Medium	Heavy	Yellow	—
		4	Too bright; mottled	Bad	Medium	Heavy	Dark	Off
Wax Dispersion B	15	5	Pink patches	Sl. off	Very sl. moist	One colony	—	Off
		6	Pink patches	Very sl. off	Very sl. moist	One colony	—	Off
	31	5	Pinkish	Bad	Slight	Medium'	Discoloured	Off
		6	Pinkish	Bad	Slight	Slight	Discoloured	Off
Dimethylol urea followed by Wax Dispersion A	15	7	Sl. light	Sl. chem.	Moist	None	Satisfactory	Satisfactory
		8	Sl. dark	Sl. chem.	Moist	None	Satisfactory	Satisfactory
	31	7	Sl. brown*; very sl. mauve	Sl. chem.	None	None	Satisfactory	Satisfactory
		8	Med. brown*; sl. mauve and dark	Sl. chem.	None	None	Satisfactory	Satisfactory
Dimethylol urea followed by Wax Dispersion B	15	9	Light mauve	Sl. chem.	Moist	None	Satisfactory	Satisfactory
		10	Light mauve	Sl. chem.	Moist	None	Satisfactory	Satisfactory
	31	9	Sl. brown*; sl. grey	Sl. chem.	None	None	Satisfactory	Satisfactory
		10	Med. brown*; sl. grey	Sl. chem.	None	None	Satisfactory	Satisfactory

*Darkening of the meat indicating slight methaemoglobin formation.

TABLE VI

RESULTS OF ORGANOLEPTIC EXAMINATION OF WILTSHIRE BACON TREATED WITH
CARBON DIOXIDE AND STORED IN LATEX BAGS AT 15.6° C.

Wt. of CO_2, gm.	Days in storage	Sample No.	Lean				Fat	
			Colour	Odour	Slime	Mould	Odour	Colour
0	15	1	Sl. bright	—	—	None	—	Sl. stained
		2	Sl. bright	—	—	None	—	Sl. stained
	30	1	Good	Sl. off	Heavy	None	Good	Sl. stained
		2	Good	Sl. off	Heavy	None	Good	Good
5	15	3	Sl. brown*	—	—	Slight	—	Good
		4	Sl. brown*	—	—	Slight	—	Good
	30	3	Sl. brown*	Off	Heavy	Slight	Good	Good
		4	Sl. brown*	Sl. off	Heavy	Slight	Good	Good
10	15	5	Bright	—	—	Medium	—	Good
		6	Bright	—	—	Slight	—	Good
	30	5	Good	Sl. off	Heavy	Slight	Good	Good
		6	Good	Sl. off	Heavy	Slight	Good	Good
15	15	7	Bright	—	—	Medium	—	Good
		8	Bright	—	—	Heavy	—	Good
	30	7	Sl. brown*	Sl. off	Heavy	Heavy	Good	Good
		8	Sl. brown*	Sl. off	Heavy	Slight	Good	Good
20	15	9	Bright	—	—	Very slight	—	Good
		10	Bright	—	—	Medium	—	Good
	30	9	Good	Bad	Heavy	Medium	Good	Good
		10	Good	Sl. off	Heavy	Medium	Good	Good

Darkening of the meat indicating slight methaemoglobin formation.

effect is presumably attributable to a lowering of the pH of the meat because of solution of carbon dioxide. The results of this and the preceding experiment indicate that carbon dioxide is not effective in preventing spoilage of bacon stored at high temperatures.

Sodium Hydroxide

In a previous experiment on the use of sodium hydroxide as a preservative for bacon (7) the compound was applied in the solid form. Because of its hygroscopic nature, however, considerable difficulty was encountered in applying and distributing the hydroxide uniformly over the meat surface. In order to eliminate these difficulties a study was made of the suitability of dipping bacon in aqueous solutions of sodium hydroxide.

The material consisted of one freshly-cured Wiltshire back divided into 10 approximately equal portions. Two pieces selected at random were dipped for 10 min. in sodium hydroxide solutions of the following concentrations: 5, 10, 20, 30, and 40%. Each sample was drained for approximately one hour, then wrapped in waxed paper, and overwrapped with brown. Organoleptic examination of the bacon was made after 15 and 30 days' storage at 15.6° C.

The results are shown in Table VII. While retarding the growth of bacteria and moulds, sodium hydroxide failed to prevent spoilage of the bacon. This is in contrast to the rather favourable results previously obtained with sodium

TABLE VII

RESULTS OF ORGANOLEPTIC EXAMINATION OF WILTSHIRE BACON DIPPED IN SOLUTIONS OF SODIUM HYDROXIDE AND STORED AT 15.6° C.

Conc. NaOH, %	Days in storage	Sample No.	Lean				Fat	
			Colour	Odour	Slime	Mould	Colour	Odour
5	15	1	Sl. grey	Sl. off	Moist	Slight	Sl. yellow	Sl. off
		2	Sl. grey	Sl. off	Moist	Slight	Sl. yellow	Sl. off
	30	1	Good	Bad	Heavy	Medium	Sl. yellow	Sl. off
		2	Good	Bad	Heavy	Medium	Sl. yellow	Sl. off
10	15	3	Sl. grey	Sl. off	Sl. moist	None	Sl. yellow	Sl. off
		4	Sl. grey	Sl. off	Very moist	None	Sl. yellow	Sl. off
	30	3	Good	Bad	Heavy	None	Sl. yellow	Sl. off
		4	Dark	Bad	Heavy	None	Sl. yellow	Sl. off
20	15	5	Sl. dark	Sl. off	Very moist	None	Sl. yellow	Sl. off
		6	Sl. dark	Sl. off	Moist	None	Sl. yellow	Sl. off
	30	5	Good	Off	Slight	None	Sl. yellow	Sl. off
		6	Good	Off	Medium	V. slight	Sl. yellow	Sl. off
30	15	7	Sl. dark	Sl. off	Moist	None	Sl. yellow	Sl. off
		8	Sl. dark	Sl. off	Moist	None	Sl. yellow	Sl. off
	30	7	Good	Bad	Moist	V. slight	Sl. yellow	Sl. off
		8	Good	Off	Medium	None	Sl. yellow	Sl. off
40	15	9	Sl. dark	Sl. off	Moist	None	Sl. yellow	Sl. off
		10	Sl. dark	Sl. off	Moist	None	Sl. yellow	Sl. off
		9	Sl. dark	Off	Moist	None	Sl. yellow	Sl. off
		10	Sl. dark	Bad	Moist	None	Sl. yellow	Sl. off

hydroxide applied in the solid form (7). It would appear that sodium hydroxide is more effective as a preservative for bacon when applied as a solid rather than as a solution.

Desiccation

The results of previous investigations have shown that smoking improves the keeping quality of bacon (4, 8, 9). It was considered that preservation might be further enhanced by partial desiccation of the bacon subsequent to smoking.

Materials and Procedure

The material consisted of 12 Wiltshire-cured backs taken from different hogs. After washing and brushing with water, the backs were hung in a small experimental smoke house, and the surface dried for two hours at a temperature of 32.1° C. (90° F.) and an air flow of approximately 500 cu. ft. per min. The air temperature was then raised to 40.6° C. (105° F.) and the backs given a relatively heavy smoke for six hours, after which drying alone was continued.

One back, selected at random, was removed for study and weighed after the following periods (from the initiation of the experiment): 8, 23, and 38 hr.; 4, 7, 8, 9, 12, 15, 17, 21, and 29 days. After removal from the house, the

front end of the back was trimmed and a slice removed for determination of the moisture content of the lean (6). The remainder of the back was wrapped in three layers of waxed paper, overwrapped with brown, and stored at 15.6° C. Organoleptic examinations of the backs and peroxide oxygen determinations on the fat (3) were made after storage for 30 and 60 days, and deep-meat bacterial counts (6) after 30 days.

Results

Data on the loss in weight, moisture content, and deep-meat bacterial counts of the bacon, and the peroxide oxygen content of the fat are given in Table VIII. Desiccation of the backs occurred very slowly, since even after treat-

TABLE VIII

EFFECT OF SMOKING AND PARTIAL DESICCATION ON THE PEROXIDE OXYGEN CONTENT AND DEEP-MEAT BACTERIAL GROWTH IN BACON STORED AT 15.6° C.

Time of treatment	Loss in weight of backs, %	Moisture content of lean meat, %	Peroxide oxygen content, ml. $0.002N$ $Na_2S_2O_3$		Deep-meat bacterial count after 30 days' storage, $\log_{10}$ no. per gm.	
			30 days' storage	60 days' storage	Large colonies	Pin-point colonies
Hr.						
8	2.4	67.7	1.2	6.1	8.77	—
23	5.4	68.7	5.0	10.3	6.19	7.33
38	7.1	64.7	13.5	11.0	6.27	0
Days						
4	10.1	64 6	1.8	5.5	8.49	0
7	19.6	60.4	5.9	4.1	4.38	0
8	18.8	55.9	5.1	5.3	3.67	0
9	22.8	58.1	6.6	5.0	5.55	0
12	24.6	56.2	5.3	2.5	0	0
15	30.5	50.9	7.7	1.5	0	0
17	37.3	49.8	9.9	11.3	3.05	0
21	42.4	35.7	10.3	12.9	3.72	0
29	53.6	27.5	22.2	4.9	2.72	0

ment for 29 days the meat still contained 27% moisture. It should also be noted that the values for loss in weight include not only moisture but fat rendered from the backs, especially at the longer drying periods. The peroxide oxygen values of the fat were usually low and somewhat variable, reflecting the antioxidant effect of smoking. Drying to moisture contents of 55 to 60% and lower markedly decreased the deep-meat bacterial counts. The absence of pin-point colonies at the lower moisture levels is considered to be of significance. In previous experiments on the preservation of bacon by hard curing, the presence and development of this type of micro-organism in bacon appeared to be related to the development of stale and off-odours (6).

Results for the organoleptic examination of the backs after storage for 30 and 60 days at 15.6° C. are given in Table IX. Spoilage in the lean meat and slime formation on the surface was prevented by drying to a moisture

TABLE IX

ORGANOLEPTIC EXAMINATION OF SMOKED AND DRIED BACON AFTER STORAGE AT 15.6° C.

Time of treat-ment	Storage period, days	Lean				Fat, odour	Remarks
		Colour	Odour	Slime	Mould		
Hr.							
8	30	—	Sl. off	—	Heavy	Good	—
	60	Good	Bad	Heavy	Heavy	—	Meat moist and firm
23	30	—	Sl. off	—	Heavy	—	—
	60	Good	Bad	Heavy	Heavy	—	Meat firm
38	30	—	Sl. off	—	Heavy	Good	—
	60	Good	Bad	Heavy	Heavy	—	Meat firm
Days							
4	30	Good	Good	Good	Heavy	Good	—
	60	Good	Good	Medium	Heavy	Good	Meat firm
7	30	Good	Excellent	—	Heavy	—	—
	60	Good	Good	—	Heavy	—	Meat slightly separated from fat
8	30	Sl. grey	Good	—	Heavy	—	—
	60	Sl. grey	Good	—	Heavy	—	Meat firm
9	30	Sl. grey	Good	—	Heavy	—	—
	60	Sl. grey	Good	—	Heavy	Good	Meat firm
12	30	Medium grey	Good	—	Medium	—	—
	60	Grey	Good	None	Heavy	Good	Meat cracked and readily separated from fat
15	30	Good	Good	—	Medium	—	—
	60	Dark	Fair	—	Heavy	Good	Meat soft, cracked and partially separated from fat
17	30	Good	Good	—	Slight	—	—
	60	—	Good	None	Slight	Good	Meat cracked and separated from fat
21	30	Brown	Good	None	None	—	—
	60	Brown	Good	None	None	Good	Meat very dry and cracked
29	30	—	—	—	—	—	—
	60	Dark	Good	None	None	Good	Meat very hard, dry and cracked

content of about 55 to 60% and lower. However, a heavy growth of moulds occurred on all backs with moisture contents greater than about 35%. The presence of so much mould was undoubtedly due in large part to the smoking treatment and could possibly be controlled by the application of a mouldicide, such as dimethylol urea. It would appear that a combination of smoking and partial drying, as described here, was comparatively effective in preserving Wiltshire bacon. It is probable that the drying period could be reduced to a more practical value by a suitable selection of drying conditions.

Acknowledgment

References

1. E. I. DuPont de Nemours and Co., Wilmington, Dela.   The R. and H. Chemicals Dept. Technical Bulletin No. A-1068.   1940.
2. Rosser, F. T., White, W. H., Woodcock, A. H., and Fletcher, D. A.   Can. J. Research, D, 20 : 57-70.   1942.
3. White, W. H.   Can. J. Research, D, 19 : 278-293.   1941.
4. White, W. H.   Can. J. Research, F, 22 : 97-106.   1944.
5. White, W. H.   Unpublished data.
6. White, W. H., Gibbons, N. E., and Thistle, M. W.   Can. J. Research, F, 23 : 213-230. 1945.
7. White, W. H., Gibbons, N. E., and Thistle, M. W.   Can. J. Research, F, 23 : 340-350. 1945.
8. White, W. H., Gibbons, N. E., Woodcock, A. H., and Cook, W. H.   Can. J. Research, D, 20 : 263-275.   1942.
9. White, W. H., Woodcock, A. H., and Gibbons, N. E.   Can. J. Research, F, 22 : 107-118. 1944.

CANADIAN WILTSHIRE BACON

XXVII. EFFECT OF METHOD OF THAWING FROZEN PORK ON BACON QUALITY[1]

By W. Harold White[2], G. A. Grant[3], and N. E. Gibbons[4]

Abstract

Frozen Wiltshire sides were defrosted at temperatures of 4.4°, 12 6°, and 21.0° C. (40°, 55°, and 70° F.) in water; curing pickle; 5, 15, and 30% brines; and in air at high and low relative humidity. Differences due to defrosting procedure were determined by measurement of the thawing period, changes in weight, content of moisture and curing salts, surface bacterial growth, peroxide oxygen formation in the fat, and colour quality and brightness of the lean meat. The effect of method of thawing on keeping quality during storage at − 1.1° C. (30° F.) was also studied.

While significant differences were observed between individual thawing treatments in the various criteria employed, few consistent trends could be distinguished between the three temperatures and the four types of media. However, in general it appeared that the more suitable procedures were those that effected thawing within a reasonable period of time. Unduly prolonged exposure to any of the conditions was usually undesirable.

Introduction

Under Canadian conditions it is usually necessary in periods of low hog production to use appreciable quantities of frozen, stored pork for the manufacture of Wiltshire bacon. Suitable conditions for frozen storage have been described previously (1, 2, 5). From two of these studies (1, 5), preliminary data were obtained on the relative suitability of thawing pork in air, water, curing pickle, and a saturated solution of sodium chloride. It was observed that thawing in pickle or brine gave pork a darker colour and more stable fat. The present paper describes a more extensive investigation on the effect of temperature, and the nature and concentration of the thawing medium on surface bacterial growth, colour of the lean meat, and development of rancidity in the fat of pork after conversion to bacon.

Material and Procedure

The right and left sides of 23 hogs were cooled and butchered according to regular commercial practice. After being wrapped with waxed paper and inserted in heavy paper bags, the sides were placed in a sharp-freezer at − 26.1° C. (− 15° F.) for 24 hr. and subsequently stored at −17.7° C. (0° F.). Two sides, selected at random, were defrosted at temperatures of

4.4°, 12.6°, and 21.0° C. (40°, 55°, and 70° F.), respectively, in each of the following solutions: 5, 15, and 30% sodium chloride; rebuilt Wiltshire cover pickle; water.   Two wrapped and two unwrapped sides also were defrosted at the same temperatures in air at an average relative humidity of 48% and, in addition, at 12.6° C. and an average relative humidity of 95%.   Systematic differences between sides due to the length of the period of frozen storage prior to defrosting were eliminated by randomizing the order in which the thawing conditions were studied.

Thawing in liquids was carried out in a small wooden tank containing about 60 gal. of the desired solution.   By means of a centrifugal pump the liquid was continuously circulated from the bottom of one end of the tank to the top of the opposite end.   It was maintained at a constant temperature by passage through a thermostatically controlled heat exchanger.   Thawing in air was accomplished by hanging the sides in a small, insulated room provided with suitable controls for temperature and relative humidity.

Temperature changes in each side during thawing were followed by thermocouples inserted to a depth of about 2 in. in each of the following three positions: in the gammon at a point 5 in. from the top edge of the side and 3.5 in. from the femur; in the back opposite the eighth rib and 2 in. from the top edge; and in the fore-end 5.5 in. anterior to the first rib and 5 in. from the top edge of the side.   Temperature readings of the sides were made at 15 to 60 min. intervals, depending on the temperature of the thawing, until all three points had reached 1.6° C. (35° F.) or higher.

After removing the sides from the thawing bath or chamber, they were weighed, drained for one day, and reweighed.   The sides were pumped with Wiltshire pump pickle by commercial operators, weighed, and cured at 4.4° C. (40° F.) for four days in small tanks containing Wiltshire cover pickle. (The short curing period was employed because of the abnormally large pickle to meat ratio associated with the use of the small tank (6)).   After removal from cure, the sides were drained for one day, weighed, and cut up to obtain the long rib-in English style backs.

A sample was removed from the rear of each back for determination of the chloride, nitrate, nitrite, and moisture content of the lean meat (4, 7); and from the front, for measurement of the colour (8, 9).   Samples (3 sq. cm.) of surface tissue, removed from each of three approximately equidistant positions along the back, were taken for bacterial counts (3).   The backs were then wrapped in waxed paper, overwrapped in brown, and stored at − 1.1° C. (30° F.).   Bacterial counts were repeated after 30 and 60 days' storage, and colour measurements of the meat and peroxide oxygen determinations on the fat after 30, 60, and 90 days.

Results

Defrosting Time

At any one temperature the time required for defrosting was approximately of the same order of magnitude in all the liquid media studied, and considerably shorter than in air of low relative humidity (Table I).   Wrapping the

TABLE I

EFFECT OF TEMPERATURE AND MEDIUM OF THAWING ON THE PERIOD REQUIRED
TO THAW FROZEN WILTSHIRE SIDES

Thawing medium	Temperature of thawing medium, °C.	Time[1], hr.	Temperature[1] (° C.) of:		
			Fore-end	Back	Gammon
Brine					
5%	4.4	24.3	3.6	6.3	1.7
	12.6	12.3	3.9	12.5	1.7
	21.0	6.9	9.0	18.6	1.7
15%	4.4	27.2	5.0	5.8	1.7
	12.6	14.8	3.0	13.1	1.7
	21.0	6.8	4.9	15.1	1.7
30%	4.4	22.3	1.9	5.6	1.7
	12.6	10.2	4.6	12.3	1.7
	21.0	5.3	2.2	15.9	1.7
Wiltshire curing pickle	4.4	28.0	2.6	4.5	1.7
	12.6	13.6	5.5	12.8	1.7
	21.0[2]	7.8	11.1	17.0	1.7
Water	4.4	21.1	2.1	3.7	1.7
	12.6	8.2	7.5	11.3	1.7
	21.0	9.3	8.1	19.6	1.7
Air, low *R. H.*	4.4	111.7	2.9	4.2	1.7
Wrapped	12.6	51.4	4.1	7.2	1.7
	21.0[2]	37.4	12.2	15.6	1.7
Unwrapped	4.4	52.1	2.7	2.8	1.7
	12.6	29.7	6.0	9.7	1.7
	21.0	24.2	15 0	11.8	1.7
Air, high *R. H.*					
Wrapped	12.6	47.5	6.3	5.4	1.7
Unwrapped	12.6	13.7	4.7	9.7	1.7

[1] *Mean values for two sides.*
[2] *Values obtained on one side only.*

sides materially lengthened the defrosting period. An increase in the relative humidity had little effect on the period required for wrapped sides at the one temperature studied, but reduced the time for unwrapped sides to about that observed for pickle, brine, or water.

With air defrosting at a low relative humidity and with water there was a marked difference between the times required at 4.4° and 12.6° C., and a considerably smaller difference between 12.6° and 21.0° C. In the other media an increase in temperature of 8.2° C. effected a reduction of about one-half in the defrosting period.

Changes in Weight

Mean values for changes in weight of the sides during thawing, pumping, and curing are given in Table II. Sides held in any of the liquids or in air of high relative humidity increased in weight during thawing, while those treated

TABLE II

EFFECT OF TEMPERATURE AND MEDIUM OF THAWING ON CHANGES IN WEIGHT OF
WILTSHIRE SIDES DURING THAWING, PUMPING, AND CURING

Thawing medium	Temperature of thawing medium, ° C.	Change in weight of sides[1], %		
		During thawing	During pumping and curing	During thawing, pumping, and curing
Brine				
5%	4.4	3.8	2.6	5.6
	12.6	3.4	3.2	6.3
	21.0	2.6	4.9	6.7
15%	4.4	3.5	2.8	6.3
	12.6	2.6	4.0	6.4
	21.0	2.6	4.4	6.8
30%	4.4	0.8	4.6	5.7
	12.6	0.6	4.7	5.5
	21.0	0.9	3.8	4.3
Wiltshire curing pickle	4.4	0.5	2.7	3.4
	12.6	1.4	4.8	6.1
	21.0	1.1	3.2	6.6
Water	4.4	3.4	6.3	9.2
	12.6	3.0	3.4	6.4
	21.0	2.4	3.6	6.2
Air, low *R. H.* Wrapped	4.4	−1.2	4.6	3.7
	12.6	−0.8	8.6	7.9
	21.0	−0.6	5.4	4.7
Unwrapped	4.4	−3.4	4.0	2.3
	12.6	−1.5	7.3	6.1
	21.0	−1.9	7.4	5.5
Air, high *R. H.* Wrapped	12.6	2.0	3.8	5.0
Unwrapped	12.6	0.2	5.4	6.0

[1] *Mean values for two sides.*

in air of low relative humidity decreased.   Increases in weight were greatest
and approximately of the same order of magnitude with the use of 5 and 15%
brine; water; and, for wrapped sides, air of high relative humidity.   For
sides defrosted in air, wrapping decreased the losses in weight occurring at the
low relative humidity and increased the gains in weight at the high humidity.
While mean differences due to defrosting temperatures were for the most
part small, the greatest changes in weight were usually associated with a
defrosting temperature of 4.4° C.   This behaviour is presumably a reflection
of temperature on the length of the thawing period.

As was to be expected, all sides increased in weight during pumping and
curing.   The increase was, in general, greatest for those conditions that
caused minimum changes during thawing.

The over-all increases in weight as a result of thawing, pumping, and curing showed no systematic differences within or between the various groups of defrosting conditions. However, when averaged over all thawing media the increase in weight was greatest at 12.6° and least at 4.4° C.

Content of Moisture and Curing Salts

Mean values for the content of moisture, chloride, nitrate, and nitrite in the back bacon after cure are given in Table III. Variations in moisture

TABLE III

EFFECT OF TEMPERATURE AND MEDIUM OF THAWING ON THE CONTENT OF MOISTURE AND CURING SALTS IN WILTSHIRE BACK BACON

Thawing medium	Temperature of thawing medium, ° C.	Component[1]			
		Moisture, %	Sodium chloride, %	Sodium nitrate, %	Sodium nitrite, p.p.m.
Brine 5%	4.4	68.2	5.56	0.13	40
	12.6	70.2	5.52	0.11	33
	21.0	68 5	4.94	0.13	67
15%	4.4	69.2	5.97	0.12	53
	12 6	69.5	4.18	0.10	34
	21.0	66.0	8.04	0.22	97
30%	4.4	68.6	5.38	0.21	53
	12.6	71.0	3.43	0.07	18
	21.0	69.0	5.47	0.08	27
Wiltshire curing pickle	4.4	69.1	5.89	0.16	42
	12 6	69.2	6 54	0.16	51
	21.0	65.1	7.01	0 21	36
Water	4.4	71.2	4.43	0.14	68
	12.6	72.0	5.07	0.11	43
	21.0	69.8.	6 01	0.18	33
Air, low *R. H.* Wrapped	4.4	68.8	4 21	0.18	54
	12.6	67.0	5 62	0.14	72
	21.0	70.1	5.95	0.20	47
Unwrapped	4.4	69.9	4.74	0.16	26
	12.6	71.8	3.38	0.11	53
	21.0	71.8	5.95	0 09	59
Air, high *R. H.* Wrapped	12.6	70.6	4.04	0.10	40
Unwrapped	12.6	76.0	6.25	0.14	92

[1] *Mean values of duplicate determinations for each of two sides.*

content showed no systematic trends between the temperatures or thawing media studied. However, the values, as averaged over all temperatures, were greatest for unwrapped sides thawed in water or in air of low relative humidity and least for those held in pickle or in air of high relative humidity.

The nature of the thawing medium had little effect on the content of curing salts, indicating that brine or curing pickle can be employed without giving an undesirably salty product. The highest concentrations of sodium chloride were usually associated with a temperature of 21.0° C. However, with this possible exception there was little to indicate the superiority of any particular defrosting condition.

Surface Bacterial Growth

Surface bacterial counts on the back bacon prior to storage were, on the average, lowest for those sides that had been defrosted in 15 or 30% brine, in curing pickle, or, unwrapped, in air of low relative humidity and a temperature of 21.0° C. (Table IV). Presumably the effect of temperature is, in large part, a reflection of the length of time required for thawing and suggests that

TABLE IV

EFFECT OF TEMPERATURE AND MEDIUM OF THAWING ON SURFACE BACTERIAL GROWTH ON WILTSHIRE BACK BACON DURING SUBSEQUENT STORAGE AT −1.1° C. (30° F.)

Thawing medium	Temperature of thawing medium, ° C.	Log_{10} number of organisms per sq. cm.[1]		
		Storage period, days		
		0	30	60
Brine 5%	4.4	6.39	6.93	8.50
	12.6	4.96	6.90	8.55
	21.0	6.36	8.92	8.33
15%	4.4	4.53	8.44	9.09
	12.6	4.28	4.66	6.79
	21.0	5.42	6.72	8.48
30%	4.4	4.32	5.44	7.60
	12.6	4.28	6.26	8.75
	21.0	4.62	7.43	9.12
Wiltshire curing pickle	4.4	5.65	6.68	8.31
	12.6	4.99	9.45	8.89
	21.0	3.46	6.41	7.80
Water	4.4	7.28	8.76	8.93
	12.6	6.20	8.90	8.89
	21.0	4.27	3.98	8.13
Air, low *R. H.* Wrapped	4.4	5.65	8.79	9.19
	12.6	5.56	8.75	9.08
	21.0	5.21	8.01	9.04
Unwrapped	4.4	4.16	7.50	9.02
	12.6	5.29	9.06	9.20
	21.0	4.59	6.55	7.98
Air, high *R. H.* Wrapped	12.6	6.48	9.10	8.58
Unwrapped	12.6	5.71	7.73	8.86

[1] *Mean values for two sides.*

this period should be kept to a minimum. Wrapped sides defrosted in air ·tended to have higher surface bacterial counts.

Surface bacterial growth occurred during storage at $-1.1°$ C.; the rate of increase was greatest during the first 30 days storage, and was relatively greater the lower the initial count. Differences between sides defrosted by the various procedures were small after storage for 60 days and were of little statistical significance.

Peroxide Oxygen Formation in the Fat

The peroxide oxygen values of the back bacon fat were variable in behaviour with respect both to the defrosting condition and the subsequent storage period at $-1.1°$ C. (Table V). However, lower peroxide oxygen values were obtained by thawing the unwrapped sides in liquid media, at the

TABLE V

EFFECT OF TEMPERATURE AND MEDIUM OF THAWING ON PEROXIDE OXYGEN FORMATION IN THE FAT OF WILTSHIRE BACK BACON DURING SUBSEQUENT STORAGE AT $-1.1°$ C. (30° F.)

Thawing medium	Temperature of thawing medium, °C.	Peroxide oxygen content[1], ml. 0.002 N Na$_2$S$_2$O$_3$		
		Storage period, days		
		30	60	90
Brine				
5%	4.4	6 0	10.2	19.9
	12.6	14.7	14.6	11.1
	21.0	21.0	16.6	24.0
15%	4 4	6.9	12.2	7.8
	12.6	20.1	27.0	10.1
	21.0	5.6	13.8	13.3
30%	4.4	30.1	33.2	16.7
	12.6	22.0	12.0	10.4
	21.0	5.2	10.0	10.2
Wiltshire curing pickle	4.4	7.9	6.5	8.8
	12.6	10.4	10.3	13.2
	21.0	40.9	26.3	25.5
Water	4.4	6.1	4.7	9.1
	12.6	21.7	19.5	13.1
	21.0	26.1	22.8	23.8
Air, low *R. H.*				
Wrapped	4.4	30.7	27.3	16.2
	12.6	7.8	5.7	8.3
	21.0	4.9	8.3	8.6
Unwrapped	4.4	6.6	10.8	8.2
	12.6	3.8	7.1	3.4
	21.0	8.2	15.5	9.7
Air, high *R. H.*				
Wrapped	12.6	11.7	4.0	10.9
Unwrapped	12.6	16.9	18.0	14.3

[1] *Mean values of duplicate determinations for each of two sides.*

lower temperatures, and wrapped sides in air of low humidity, at the higher temperatures; these conditions would, therefore, seem to be more suitable.

In a previous investigation (5), in which small cuts of pork were used, it was observed that defrosting in saturated brine or pickle at 4.5° C. usually yielded a product with slightly less peroxide oxygen than did thawing in air at 10.0° C. or water at 38.0° C. The present results are in substantial agreement with these previous conclusions when consideration is given to the differences in experimental conditions employed.

Colour Brightness and Quality

Mean values for colour brightness are given in Table VI. Neither temperature nor thawing medium had significant effect on colour brightness. A general and statistically significant increase in brightness occurred during storage at − 1.1° C.

TABLE VI

EFFECT OF TEMPERATURE AND MEDIUM OF THAWING ON CHANGES IN COLOUR BRIGHTNESS OF
WILTSHIRE BACK BACON DURING SUBSEQUENT STORAGE AT −1.1° C. (30° F.)

Thawing medium	Temperature of thawing medium, ° C.	Total scatter[1], %	
		Storage period, days	
		0	90
Brine			
5%	4 4	11.8	13.0
	12 6	10 3	11.8
	21.0	10.9	12.2
15%	4 4	11.7	13.3
	12.6	11 4	11.5
	21.0	12 9	13.6
30%	4 4	11 4	11.8
	12.6	14 5	12 4
	21.0	11.5	12.0
Wiltshire curing pickle	4.4	11.9	12.3
	12.6	11.2	12 7
	21.0	11.0	11.9
Water	4 4	10.5	12.7
	12 6	10.4	12.2
	21.0	12.4	12.6
Air, low *R. H.*			
Wrapped	4.4	12.5	13.4
	12.6	12.0	12.1
	21.0	10.4	12.4
Unwrapped	4.4	10.4	11.3
	12.6	13.9	13.9
	21.0	10 7	12 6
Air, high *R. H.*			
Wrapped	12.6	12.8	12.0
Unwrapped	12.6	12.7	13.4

[1] *Mean values of duplicate determinations for each of two sides.*

While data were obtained for nine colour bands (9), statistical analyses indicated that the method of defrosting had little significant effect on colour quality. Accordingly, the data have been grouped for more ready comparison of over-all changes in the blue, green, and red spectral regions (Table VII). During the storage at $-1.1°$ C., the light scattered in the blue region decreased; green scatter increased; while red scatter decreased after 30 days and subsequently increased.

TABLE VII

EFFECT OF TEMPERATURE AND MEDIUM OF THAWING ON COLOUR QUALITY OF WILTSHIRE BACK BACON DURING SUBSEQUENT STORAGE AT $-1.1°$ C. (30° F.)

Thawing medium	Tempera-ture of thawing medium, °C	Mean scatter[1], %					
		Blue, 3850–5250 Å		Green, 5250–5840 Å		Red, 5840–6440 Å	
		0 days	90 days	0 days	90 days	0 days	90 days
Brine 5%	4.4	48.8	38.6	25.5	28.7	25.7	32.7
	12.6	45.7	43.1	27.7	28.1	26.6	29.0
	21.0	46.2	43.2	26.4	29.1	27.6	28.1
15%	4.4	47.7	39.0	25.8	28.6	26.6	32.4
	12.6	47.6	46.8	27.4	26.7	25.1	27.1
	21.0	48.4	40 9	26.3	28.1	25.5	31.1
30%	4.4	44.3	45.2	26.8	28.1	28.9	26.8
	12.6	41.1	44.0	29.1	28.8	30.0	27.3
	21.0	46.5	42.0	25.7	29.2	27.9	28.9
Wiltshire curing pickle	4.4	47.8	41.9	26.0	27.5	26.2	30.7
	12.6	47.6	41.0	26.3	30.3	26.5	28.8
	21.0	45.6	47.0	27.3	27.0	27.2	26.2
Water	4.4	47.1	41.6	27.0	28.6	25.9	29.9
	12.6	47.7	41.3	25.3	29.2	27.7	29.7
	21.0	48.4	46.4	26.9	27.1	24.7	26.6
Air, low R. H. Wrapped	4.4	44.2	44.4	27.7	28.9	28.2	26.9
	12.6	45.9	42.7	27.7	28.7	26.6	28.7
	21.0	45.9	40.3	26.7	28.1	27.3	31.6
Unwrapped	4.4	48.1	42.5	25.6	28.4	26.5	29.7
	12.6	46.8	39.3	26.8	30.7	26.6	32.9
	21.0	46.3	40.3	26.3	27 3	27.6	32.5
Air, high R. H. Wrapped	12.6	46.7	42.2	26.7	28.6	26.6	28.9
Unwrapped	12.6	48.0	42.7	26.7	28.6	25.5	28.4

[1] *Mean values of duplicate determinations for each of two sides.*

Conclusions

Under the conditions employed in this study no one thawing medium appeared to be markedly superior to the others. In air, wrapping prolonged, but a high relative humidity accelerated, the defrosting. Thawing in all the liquid media occurred at approximately the same rate. Increases in weight during thawing were greater in 5 than 30% brine or in curing pickle. The

smallest defrosting gains or losses were associated with a temperature of 21.0° C. After cure there was little difference in weights between sides defrosted by the various procedures. However, there was some indication that, on the average, a temperature of 12.6° C. and water or brine of 5 or 15% concentration favoured maximal increases in weight. While the various defrosting media had no marked effect on the content of moisture and curing salts, a higher thawing temperature tended to give a somewhat saltier product.

The surface bacterial counts tended to be somewhat lower when the sides were defrosted at 21.0° C. in water, curing pickle, or the more concentrated brines. The highest mean count was obtained on sides defrosted in water at 4.4° C. In air either wrapping or a high relative humidity enhanced surface bacterial growth. Defrosting conditions likewise had little specific effect on peroxide oxygen formation in the fat. In general a low temperature was more suitable for the liquid media, and a high temperature for air at low relative humidity with wrapped sides. The defrosting methods studied appeared to have no systematic influence on either colour, quality, or brightness.

While the somewhat indefinite character of the results precludes the possibility of definite conclusions, there is some indication that liquid media or air at high humidity may possibly be superior to dry air and that a defrosting temperature of 12.6° C. merits commercial trial.

Acknowledgments

References

1. Cook, W. H. Can. J. Research, D, 19 : 85-95. 1941.
2. Cook, W. H. and White, W. H. Can. J. Research, D, 19 : 53-60. 1941.
3. Gibbons, N. E. Can. J. Research, D, 18 : 202-210. 1940.
4. White, W. H. Can. J. Research, D, 17 : 125-136. 1939.
5. White, W. H. Can. J. Research, D, 19 : 96-103. 1941.
6. White, W. H., Gibbons, N. E., Woodcock, A. H., and Cook, W. H. Can. J. Research, D, 20 : 263-275. 1942.
7. White, W. H., Winkler, C. A., and Cook, W. H. Can. J. Research, D, 19 : 213-224. 1941.
8. Winkler, C. A., Cook, W. H., and Rooke, E. A. Can. J. Research, D, 18 : 435-441. 1940.
9. Woodcock, A. H. Can. J. Research, D, 21 : 90-97. 1943.

general quality as compared to samples prepared from commercial shortenings. The grading is done by a panel, usually 10 experienced judges.

Experimental

Small samples of 250-275 g. of commercial alkali-refined linseed oil were subjected to the heat polymerization process at various temperatures from 260-300° C. for periods up to 30 hours. The results presented in Figure 1 show that in the earlier stages of iso-
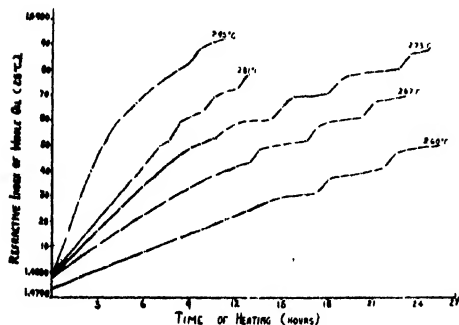
FIG. 1. Polymerization of alkali-refined linseed oil at various temperatures.

thermal polymerization a linear relationship exists between the refractive index and the time of heated whereas in the later stages the reactions proceed in a stepwise manner. This is especially apparent at the lower temperatures when the reaction rate is slower.

The temperature at which the polymerization process is carried out is especially important in obtaining the desired oil for the preparation of shortening. If the polymerization is carried out at temperatures above 280°C. or at temperatures so low that the period of heating is unduly prolonged, the shortenings obtained are of poor quality. Some improvement is obtained by using the acetone soluble fraction of these polymerized oils, but they still do not make good quality shortenings. *Our experience indicates that the best oil is obtained by heating at 270-275°C. for 12-15 hours. At this stage (Fig. 1) the whole oil has a refractive index of 1.4858-1.4861 and yields 60-65% of acetone soluble oil with a refractive index of 1.4830-1.4834.*

The continuous passage of carbon dioxide through the oil is an essential condition in the process as vola-

tile decomposition products including free acids are eliminated in this manner. The product is characterized by a low acid value (0.5-1% as oleic acid) and a very pale yellow color. The color of the product is a criterion of the efficiency with which the decomposition products have been removed. The volatile products comprise about 5% of the original oil. The results obtained with nitrogen have not been as good as with carbon dioxide. If the oil is simply heated under carbon dioxide, the acid value of the product may be as high as 12% (as oleic acid), and obviously it will contain all the decomposition products. The shortenings obtained from such oils are of very poor quality.

A small portion, about 4%, of the polymerized oil is soluble in 95% methanol at room temperatures. This oil has a fishy odor and a deep brown color. It has a relatively low refractive index (1.4778 at 25° C.) and a low acid value of about 0.7%, as oleic acid. Insofar as the quality of the shortening is concerned, no apparent advantage accrues from separating this fraction prior to acetone segregation.

No special process is required for the hydrogenation; all of the commercial nickel catalysts which we have tried gave satisfactory results. The course of hydrogenation at various temperatures, as indicated by refractive indices, is illustrated in Figure 2. These
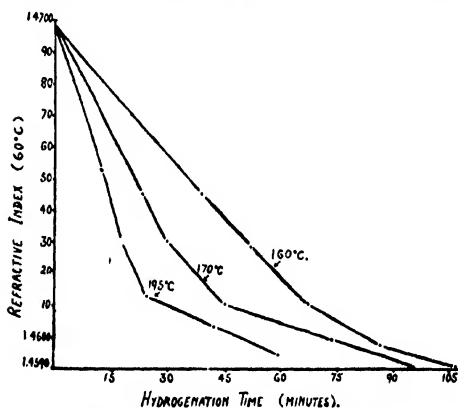
FIG. 2. Hydrogenation at various temperatures of the acetone soluble fraction of polymerized linseed oil.

results were obtained by the procedure described above and using our own catalyst, i.e. reduced nickel on silica-gel base. The best shortenings were obtained

TABLE I.

Summary of Experimental Data on the Processing of Linseed Oil.

(Alkali refined linseed oil, refractive index 1.4795, polymerization at 272-275°C. for 14 hours; hydrogenation at 170°C. to different refractive indices.)

Run No.	Whole oil R index (25°C.)	Polymerized oils				Shortenings			Judgment of pastry	
		Acetone soluble		Acetone insoluble		R. index (60°C.)	Color[3]	Consistency	Flavor reversion	Grade compared to controls[4]
		Yield[1]	R. index (25°C.)	Yield[1]	R index (25°C.)					
1	1.4859	60	1.4830	32	1.4890	1.4621	Slight yellow	Too soft	None[3]	Not as good
2	1.4860	62	1.4834	33	1.4892	1.4619	Very slight yellow	Soft	None[3]	No discernible difference
3	1.4859	63	1.4832	32	1.4889	1.4612	Pure white	Good	None[3]	No discernible difference
4	1.4858	63	1.4831	32	1.4890	1.4608	Pure white	Good	None[3]	Better than controls
5	1.4859	62	1.4830	33	1.4886	1.4604	Pure white	Slightly hard	None[3]	No discernible difference

[1] Percentage of original oil.
[2] Not decolorized with adsorbent clays nor creamed.
[3] The decision of the panel was unanimous.
[4] Two commercial samples of shortening included as standard of reference. The pastry was judged simultaneously for flavor, odor and texture by a panel of 10 judges made up of graduate dieticians, housewives, and cooks.

by hydrogenating to a refractive index of 1 4615-1 4605 (60°C.)

The data obtained in five typical runs is summarized in Table I. Preliminary trials indicate that the digestibility of these linseed shortenings, as tested with rats, compares favorably with commercial shortenings. However, further experiments are planned to assess more accurately the relative nutritive values

The high polymer, acetone insoluble fraction comprises about 30-35% of the polymerized oil (Table I) and has a refractive index of about 1 4890 at 25° C. This should make an excellent paint and varnish oil since it represents the most highly unsaturated fraction in linseed oil.

General Discussion

The aim of this investigation has been to establish the optimum conditions of polymerization and solvent segregation of linseed oil for the production of a "non-reverting" edible shortening and an improved drying oil The presence of linolenic acid in the edible fraction is most undesirable whereas it is obviously advantageous to have a high content in the drying oil fraction Therefore the conditions of polymerization should be such as will permit the highest degree of selectivity in the solvent segregation.

Time of heating	Ref active index of whole oil	Yield of acetone soluble oil	Refractive index of acetone soluble oil
hours		%	
1	1 4824	100	
1 ½	35	100	
4	59	95	1 4850
6	72	78	1 4850
8	80	60	1 4850
10	88	49	1 4850

The nature of the curves in Figure 1 indicate that the reactions involved in heat polymerization take place in a definite sequence which is most apparent in the results obtained at the lower temperatures The theoretical implications of these results may be more apparent when data are available on the chemical composition of the oil at the various stages of polymerization. However, it is well established that conjugation precedes polymerization and that conjugate trienoic acids have a higher refractive index than their polymers (2, 3, 5). The initial rise in the refractive index suggests conjugation and the flat portions of the curves may represent a state of equilibrium where intramolecular rearrangement is taking place prior to polymer formation. One advantage of heating at the lower temperatures is that polymerized oils of uniform composition are most readily obtained since the "steady state" may persist for as long as two to three hours.

These results also suggest that the polymerization is more selective at the lower temperatures. This is supported by the observation that when two samples of linseed oil are polymerized at different temperatures but to the same refractive index, the acetone soluble fraction of the sample polymerized at the lower temperature has a lower refractive index. For example, at 272-275° C. when the refractive index of the whole oil is 1,4859, 60-65% is soluble in acetone and the refractive index of this fraction is 1.4832; whereas at 295-300° C. and at the same refractive index for the whole oil, 95% is soluble in acetone and the refractive index of this fraction is 1.4850. The latter results are included in Table II which also shows that the refractive index of the acetone soluble fraction reaches a maximum thus indicating that there is a fixed limit of polymer aggregate which is soluble in acetone.

It seems a reasonable assumption that under the conditions of polymerization employed in this process the major change in the linseed oil is in the linolenic acid component which is more readily polymerized than linoleic or oleic acid. It is not known to what extent the acetone soluble oil has undergone chemical change However, it is probable that the linolenic acid in this fraction is at least changed to a conjugated isomer. This would account for the refractive index of the acetone soluble oil being slightly higher than that of the original linseed oil. This point is being investigated by conducting detailed analysis of the acetone soluble fraction.

Summary

The optimum conditions have been established for the high temperature polymerization and solvent segregation of linseed oil to produce a "non-reverting" edible shortening and an improved drying oil. The best oil is obtained by heating at 270-275° C. for 12-15 hours while carbon dioxide is continuously passed through the oil. Under these conditions the polymerized oil has a refractive index of 1.4858 to 1 4861 at 25° C and yields 60-65% of acetone soluble oil with a refractive index of 1.4830 to 1.4834 at 25° C. and an acid value of less than 1%, calculated as oleic acid. Pie crusts containing shortenings made from the acetone soluble fraction of the oil have been judged to be of good quality. The best shortenings were obtained by hydrogenating to a refractive index of 1 4615-1.4605 (60° C.).

REFERENCES

1 Armstrong, J. G., and McFarlane, W D, Oil and Soap, 21 522 7 (1944).

2. Ault, W. O., Cowan, J. C., Kass, J P and Jackson J E Ind Eng. Chem., 34, 1120 3, (1942).

3 Bradley, T F., and Johnston, W. B, Ind Eng Chem 32, 802 9, (1940).

4. Lemon, W. H., Can J. of Res, 22, 191 6, (1944).

5. Scheiber, J., Farbe u. Lack., 585 7, (1929)

A NOTE ON THE PRODUCTION OF VITAMIN C BY SPROUTING SEEDS[1]

L. P. V. Johnson, G. A. Young, and J. B. Marshall

National Research Laboratories, Ottawa

A NOTE ON THE PRODUCTION OF VITAMIN C BY SPROUTING SEEDS[1]

L. P. V. JOHNSON[2], G. A. YOUNG,[3] AND J. B. MARSHALL[2]

National Research Laboratories, Ottawa

The antiscorbutic value of germinated seeds has been known since the time of Captain Cook, and was investigated in Norway prior to the last war (5). After the Mesopotamian campaign of 1915–16 British workers directed attention to the possibility of utilizing this property as a source of accessory dietary factors for troops separated from supplies of fresh food and for the relief of famine stricken areas (1). Advances since 1918 in the knowledge of the chemistry of vitamins have made it possible to investigate more comprehensively methods for the production and preparation of an antiscorbutic material from sprouted seeds. In this note the results are given of a survey to determine the suitability, for this purpose, of seeds readily available in Canada, as well as some others less commonly known.

Legumes have been considered more prolific producers of ascorbic acid than cereals (5) and there is evidence of physiological differences between crop varieties as well. Bonner and Bonner (3) showed that pea varieties could be grouped according to their response to the presence of ascorbic acid in the media in which excised embryos were cultured. This response was related inversely to the concentration of ascorbic acid initially present in the embryos. The presence of ascorbic acid in actively growing tissues, i.e. hypocotyl, and later in chlorophyll bearing tissues has been demonstrated by Reid (4).

MATERIALS

The material examined included varieties of the following crops: common and garden peas, *Pisum sativum;* common bean, *Phaseolus vulgaris;* green gram bean[4], *P. aureus*; lima bean, *P. lunatus*; English broad bean, *Vicia fabia;* soy bean, *Glycine soja*; spring wheat, *Triticum sativum*; hullless oats, *Avena nuda*; corn, *Zea mays, v. saccharata*; okra, *Hibiscus esculentus*. A complete list including variety names is given in Table 1. The seed used in each case was of good quality, having been graded previously for seed purposes. This initial quality is of importance as sound viable seed gives some assurance of optimum performance in the sprouting tests. Broken and disease infected seeds tend to decompose readily and this may render the odour of an entire batch of seed offensive.

METHODS

Preliminary tests of the above material were made by germinating the seeds in petri dishes at 22.2° C. (72° F.) after soaking for 24 hours in tap

TABLE 1.—VARIETIES USED IN PRELIMINARY TESTS OF ASCORBIC ACID PRODUCTION

Common pea, Pisum sativum
   Field varieties
      Arthur
      Chancellor
      Dashaway
      Early Blue (Ottawa No. 21)
      Early Britain
      Golden Vine (1)*
      Mackay (Ottawa No. 25) (1)
      New Canadian Beauty
      Potter
      Prussian Blue (1)

   Garden varieties
      Dwarf Gray Sugar
      Little Marvel
      Prince of Wales (1)
      Thomas Laxton

Soy bean, Glycine soja
   Blackeye (1) (2)
   Mandarin

Okra, Hibiscus esculentus
   White Velvet (3)

Common bean, Phaseolus vulgaris
   Common white (1)
   Marrow fat (1)
   Red Kidney (1)

Lima Bean, P. lunatus
   Burpee Bush Lima (1)
   Henderson Bush Lima (1)

Green Gram bean, P. aureus
   Chinese Salad

English Broad bean, Vicia fabia
   Broad Windsor

Spring wheat, Triticum sativum
   Marquis
   Regent (2)
   Thatcher (2)

Hull-less oats, Avena nuda
   Laurel

Corn (maize), Zea mays v. saccharata
   Golden Bantam

* Reasons for elimination from further tests:
   (1) Soured during germination.
   (2) Low vitamin C production.
   (3) Poor germination.

water. Ascorbic acid determinations were made at daily intervals, commencing when the sprouts first appeared and continuing until the assay values began to decline. A number of varieties were eliminated on this basis.

The material thus selected was tested at three temperatures, 15.6°, 22.2°, and 28.9° C. (60°, 72°, and 84° F.) and by two methods of germination. In one, the seeds were soaked in trays for 24 hours, drained, spread to a depth of $\frac{3}{4}$ inch and flushed with water at the temperature required. The other method was similar to that recommended for use by the Australian Forces (6); seed was placed in loose cheese cloth bags, soaked 24 hours and thereafter dipped 4 times daily in water held at the germination temperatures.

Vitamin C assays were made on samples of the entire sprouted material. No attempt was made to separate the sprouts from the seeds and seed coats. Reduced ascorbic acid was determined by the dye titration method, 2% metaphosphoric acid being used to extract the material in a *Waring Blendor*. Moisture determinations were made on the material as well, in order to allow for the diluting effect of the tissue water. The results are expressed as mg. ascorbic acid per 100 gm. of sprouted material.

RESULTS

In the preliminary tests 14 of the 31 varieties listed in Table 1 were eliminated for various reasons. A number of the pea and bean varieties

showed a pronounced tendency to become sour and offensive when germinated in this manner.   Two of the wheat varieties were dropped because of low vitamin C production, and tests of okra were discontinued because of poor germination of the seed used.

For convenience and expediency the bag method of germination was used for most of the tests, and the data presented are based on this method. The two methods did not differ appreciably.   Considerable care was required with the trays that were used, to avoid souring as a result of impeded drainage and lack of aeration.   Trays with perforated bottoms would have been much more satisfactory.

Data showing the effects of temperature on vitamin C production and the condition of the sprouted material are given in Table 2.   Rapid souring or spoilage of all the material except the Chinese Salad bean resulted at 28.9° C.   Of the other temperatures the lower (15.6° C.) was more favourable for the legumes and the higher (22.2° C.) for the cereals.

TABLE 2.—EFFECT OF TEMPERATURE ON THE VITAMIN C PRODUCTION AND GROWTH OF SEEDS OF SELECTED VARIETIES GERMINATED IN BAGS

Crop	Variety	Temp. °C.	No. days for maximum production	*Growth	Condition of material	Mg./100 gm. of material		Percentage moisture
						Wet	Dry	
Bean	Chinese Salad	15.6	3	Good	Fresh	23	89	74
	English Broad Windsor		7	Very good	Slightly sour	33	122	73
	Mandarin Soy		4	Fair	Fresh	4	13	68
Corn	Golden Bantam		7	Good	Sour	7	20	63
Oats	Laurel		6	Very good	Fresh	8	30	75
Pea	Arthur		5	Very good	Fresh	17	61	67
Wheat	Marquis		5	Very good	Fresh	4	16	75
Bean	Chinese Salad	22.2	2	Very good	Fresh	21	65	68
	English Broad Windsor		4	Good	Sour	23	73	69
	Mandarin Soy		3	Poor	Sour	2	3	32
Corn	Golden Bantam		4	Very good	Fresh	10	30	65
Oats	Laurel		6	Very good	Slightly sour	11	19	43
Pea	Arthur		3	Fair	Fresh	3	7	64
Wheat	Marquis		4	Very good		5	13	65
Bean	Chinese Salad	28.9	2	Fair	Fresh	18	64	73
	English Broad Windsor		2	Poor	Sour	—	—	—
	Mandarin Soy		2	Poor	Sour	—	—	—
Corn	Golden Bantam		2	Poor	Sour	—	—	—
Oats	Laurel		2	Poor	Sour	1	1	43
Pea	Arthur		2	Poor	Sour	—	—	—
Wheat	Marquis		2	Fair	Slightly sour	1	2	47

* Growth poor, less than 50% germination; fair, less than 75% germination; good, less than 95% germination; very good, 95 to 100% germination.

A comparison of 12 varieties of peas sprouted in bags at 15.6° C. is given in Table 3.   Early Blue (Ottawa No. 21), O.A.C. No. 181 and Arthur

TABLE 3.—VITAMIN C PRODUCTION AND GROWTH IN FIELD AND GARDEN PEAS, SPROUTED IN BAGS AT 15.6° C.

Variety	No. days for maximum production	Growth	Mg./100 gm. of material wet basis
Arthur	5	Good	17
Chancellor	4	Poor	8
Dwarf Grey Sugar	4	Fair	11
Dashaway	4	Fair	12
Early Blue, Ottawa No. 21	5	Good	20
Early Britain	4	Fair	11
Little Marvel	4	Fair	14
New Canadian Beauty	4	Poor	10
O.A.C. No. 181	5	Good	18
Potter	4	Good	13
Thomas Laxton	4	Good	10
White Wonder	4	Fair	9

took 5 days to reach their maximum vitamin C production (17 to 20 mg. per 100 g.), while the other varieties began to decline at 4 days, after having reached a maximum of 8 to 14 mg. per 100 g.

In Figure 1 the daily increases in the ascorbic acid content of 100 gm. of sprouting material are shown for selected seeds, germinated in bags at 15.6° C. The Chinese Salad bean reached a maximum in 3 days while the Broad Windsor bean took 7 days and the Early Blue pea 5 days. Mandarin soy bean and Laurel oats did not approach the level of the others but were included for comparison.

CONCLUSIONS

Of the seeds tested in the present investigation, the Chinese Salad bean appeared to have the greatest range of adaptability combined with rapid production of vitamin C. However it is not available in sufficient quantities to warrant its recommendation for use on a large scale. The field pea varieties, Early Blue (Ottawa No. 21), O.A.C. No. 181, and Arthur produced appreciable amounts of antiscorbutic in 5 days and are readily available. The English Windsor broad bean is in less favourable supply but showed considerable merit with respect to vitamin C production, particularly at the lowest temperature.

The method of germination to be employed will be determined by circumstances and should be investigated in greater detail. Experience gained from the present· experiments indicated the desirability of temperatures between 15 and 22° C. (60° and 72° F.) and of importance of drainage and aeration for the germinating material.

ACKNOWLEDGMENTS
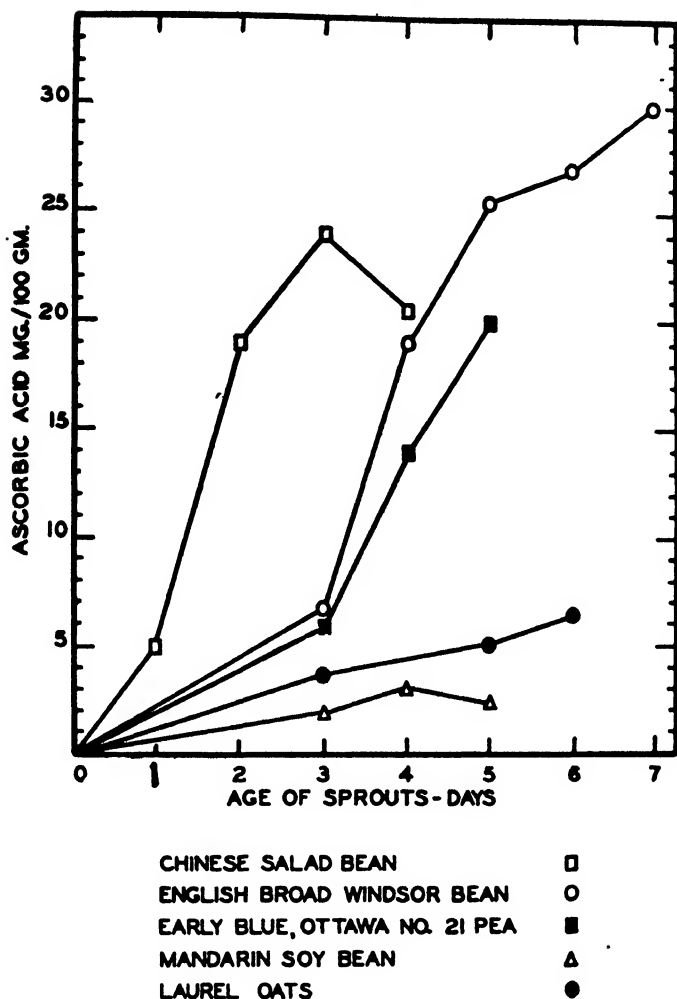
Figure 1.—Vitamin C production of selected seeds at 15.6°C. (60°F.)

REFERENCES

1. CHICK, H. and E. M. DELF. The antiscorbutic value of dry and germinated seeds. Biochem. J. 13 : 199-218. 1919.
2. BEESKOW, H. C. Bean sprouts: their preparation and properties. Mich. State College Agric. Expt. Station Tech. Bull. 184. 1943.
3. BONNER, J. and D. Bonner. Ascorbic acid and the growth of plant embryos. Proc. Nat'l Acad. Sci. U.S. 24 : 70-75. 1938.
4. REID, M. E. Localization of ascorbic acid in the cowpea plant at different periods of development. Am. J. Botany, 24 : 445-447. 1937.
5. SHERMAN and SMITH, The Vitamins, 2nd Edition. The Chemical Catalogue Co. 1931.
6. Council for Industrial and Scientific Research, Nutrition Laboratory, University of Adelaide, 1942. "The production of antiscorbutic by germinating peas".

LEGUME AND CEREAL SPROUTS AS A DIETARY SUBSTITUTE FOR FRESH VEGETABLES

W. A. Andreae, Edith A. Chalmers, and W. D. McFarlane

Macdonald College, (McGill University) Quebec

LEGUME AND CEREAL SPROUTS AS A DIETARY SUBSTITUTE FOR FRESH VEGETABLES[1]

W. A. ANDREAE[2], EDITH A. CHALMERS[2], AND W. D. McFARLANE[3]

Macdonald College, (McGill University) Quebec

Cereal and legume sprouts have been used as food for many centuries in the Orient, and records also exist which show that the early explorers cured or prevented scurvy by eating sprouted peas and beans when fresh vegetables were not available. Soybean sprouts have recently received much publicity in the United States as the result of investigations by Professor C. M. McCay of Cornell University.

Dry seeds, in general, are devoid of ascorbic acid, but the seedlings contain a considerable amount. Burkholder (1) reports an appreciable increase in riboflavin and other members of the vitamin B-complex during the growth of corn, wheat, oats and barley seedlings. Sprouts have a flavour and nutritive value which approximates that of fresh vegetables and in addition, possess several advantages in that the seeds can be sprouted, ready for use in less than 10 days and their cultivation is not limited by season, climate, sunlight or soil. The only requirements are a fairly constant room temperature of about 70° F. and a good water supply.

It might be possible to supply a significant part of the daily requirements for ascorbic acid and vitamin B-complex by the use of sprouts in the diet and hence contribute to the solution of nutritional problems arising out of the difficulty in transporting fresh vegetables to troops in isolated areas and to the population of countries released from enemy occupation. Fresh vegetables are perishable and bulky and require rapid transportation and adequate storage facilities, whereas dry seeds may be shipped to the desired location and sprouted at their destination.

The primary aims of the investigations recorded below were *(a)* to devise simple and inexpensive methods for producing sprouts in bulk, even under adverse conditions; *(b)* determine the best time to harvest for highest vitamin content commensurate with palatability; and *(c)* prepare appetizing dishes containing sprouts.

MATERIALS AND METHODS

Sprouting

Good sprouts can only be obtained from good seed; broken seeds must be eliminated as they will constitute a centre for infection. Disinfectants, such as bleaching powder, at the low concentration of 1 tablespoon to 4 gallons of water, will destroy surface bacteria without harming the seed but will not prevent all bacterial growth; equally effective results were obtained with an abundance of fresh water. The sprouting was

carried out in a basement room with two small windows, a well-drained cement floor, and a good water supply.   There was no provision for control of the room temperature which fluctuated from 66 to 73° F.

The seeds were soaked for 24 hours and held at a high humidity and a temperature of about 22° C.   The most suitable container was found to be a 6-gallon lard tub with small holes in the bottom for drainage.   The tub was half-filled with soaked seeds and covered with a lid to exclude light and to maintain a high humidity inside.   The tub was filled twice daily with water and the seeds stirred by hand to break up the sprouting mass.   The water drained through the holes in the bottom, leaving the sprouts moistened, washed and cooled.   For smaller quantities, wide-mouthed fruit jars were used.   The soaked seeds were placed in the jar and a piece of cheese-cloth was tied over the opening.   The jar was filled with water twice daily, inverted to drain and allowed to remain in this position until the next watering.

These methods were not suitable for the cultivation of green shoots. In this case the soaked seeds were spread thinly on flat wire-screen trays, 14″ × 22″, supported at each corner by wooden legs, 7″ high.   These trays (Figure 1) are light and easy to handle and can be stacked to facilitate
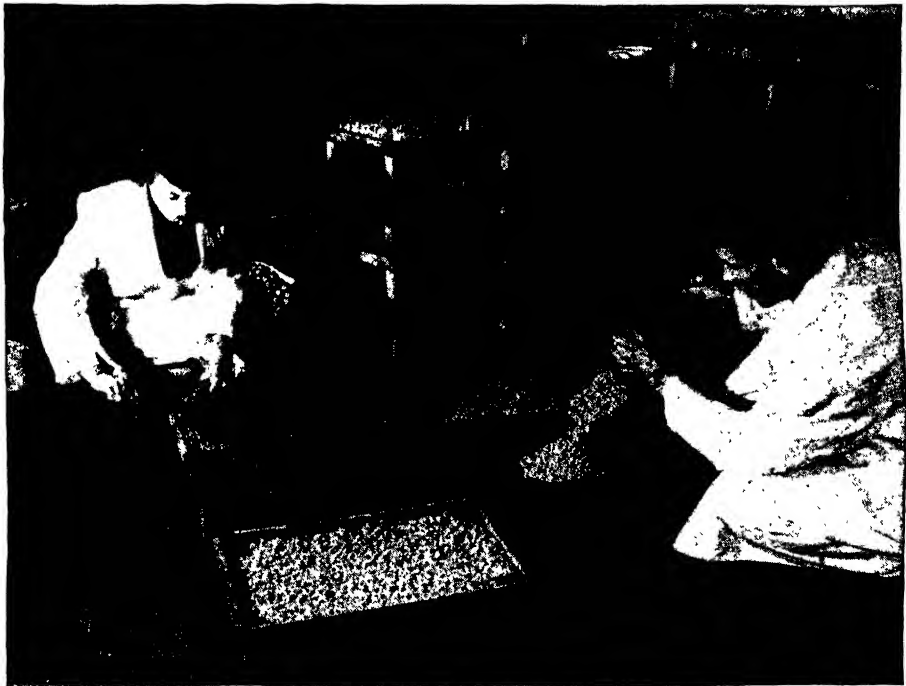

FIGURE 1.   Tray method of cultivating green shoots.

watering and utilize space.   A layer of wood shavings on the screen increased the moisture retention and facilitated the cleaning of the trays after the shoots had been cut.

LEGUME AND CEREAL SPROUTS AS A DIETARY SUBSTITUTE FOR FRESH VEGETABLES[1]

W. A. Andreae[2], Edith A. Chalmers[2], and W. D. McFarlane[3]

Macdonald College, (McGill University) Quebec

Cereal and legume sprouts have been used as food for many centuries in the Orient, and records also exist which show that the early explorers cured or prevented scurvy by eating sprouted peas and beans when fresh vegetables were not available. Soybean sprouts have recently received much publicity in the United States as the result of investigations by Professor C. M. McCay of Cornell University.

Dry seeds, in general, are devoid of ascorbic acid, but the seedlings contain a considerable amount. Burkholder (1) reports an appreciable increase in riboflavin and other members of the vitamin B-complex during the growth of corn, wheat, oats and barley seedlings. Sprouts have a flavour and nutritive value which approximates that of fresh vegetables and in addition, possess several advantages in that the seeds can be sprouted, ready for use in less than 10 days and their cultivation is not limited by season, climate, sunlight or soil. The only requirements are a fairly constant room temperature of about 70° F. and a good water supply.

It might be possible to supply a significant part of the daily requirements for ascorbic acid and vitamin B-complex by the use of sprouts in the diet and hence contribute to the solution of nutritional problems arising out of the difficulty in transporting fresh vegetables to troops in isolated areas and to the population of countries released from enemy occupation. Fresh vegetables are perishable and bulky and require rapid transportation and adequate storage facilities, whereas dry seeds may be shipped to the desired location and sprouted at their destination.

The primary aims of the investigations recorded below were *(a)* to devise simple and inexpensive methods for producing sprouts in bulk, even under adverse conditions; *(b)* determine the best time to harvest for highest vitamin content commensurate with palatability; and *(c)* prepare appetizing dishes containing sprouts.

MATERIALS AND METHODS

Sprouting

Good sprouts can only be obtained from good seed; broken seeds must be eliminated as they will constitute a centre for infection. Disinfectants, such as bleaching powder, at the low concentration of 1 tablespoon to 4 gallons of water, will destroy surface bacteria without harming the seed but will not prevent all bacterial growth; equally effective results were obtained with an abundance of fresh water. The sprouting was

carried out in a basement room with two small windows, a well-drained cement floor, and a good water supply.   There was no provision for control of the room temperature which fluctuated from 66 to 73° F.

The seeds were soaked for 24 hours and held at a high humidity and a temperature of about 22° C.   The most suitable container was found to be a 6-gallon lard tub with small holes in the bottom for drainage.   The tub was half-filled with soaked seeds and covered with a lid to exclude light and to maintain a high humidity inside.   The tub was filled twice daily with water and the seeds stirred by hand to break up the sprouting mass.   The water drained through the holes in the bottom, leaving the sprouts moistened, washed and cooled.   For smaller quantities, wide-mouthed fruit jars were used.   The soaked seeds were placed in the jar and a piece of cheese-cloth was tied over the opening.   The jar was filled with water twice daily, inverted to drain and allowed to remain in this position until the next watering.

These methods were not suitable for the cultivation of green shoots. In this case the soaked seeds were spread thinly on flat wire-screen trays, 14″ × 22″, supported at each corner by wooden legs, 7″ high.   These trays (Figure 1) are light and easy to handle and can be stacked to facilitate

FIGURE 1.   Tray method of cultivating green shoots.

watering and utilize space.   A layer of wood shavings on the screen increased the moisture retention and facilitated the cleaning of the trays after the shoots had been cut.

A portable container suitable for sprouting all types of seeds was made from a piece of coarse jute 40" × 36" (Figure 2). The bottom of this bag measured 15" × 19" and the sides were 6" high. Four sticks, less than 1" in diameter, were pushed through the open hems around the bottom, giving the bag considerable rigidity. Each corner was provided with a sling by

FIGURE 2. Portable container for sprouting all types of seeds

which the bag could be suspended by two poles or hooked to some convenient device. The bag was rendered rot proof by first immersing it in a.2% copper sulphate solution, then in 5% sodium carbonate solution and finally rinsing well with water. Rot proofing had no detrimental effect on the sprouting.

The seeds used were selected on the basis of *(a)* availability in Canada, *(b)* ease of sprouting, *(c)* nutritive value of the sprouts and *(d)* palatability of the cooked product.

Cooking

The authors considered as valuable any sprouts which were palatable and would serve to break the monotony of a diet depleted in fresh vegetables. However, such a diet is also likely to be low in vitamin C so the authors preferred to utilize sprouts of a high ascorbic acid content. Meals which included sprouts were prepared and served in the Macdonald College dining room. The final judgments of the palatability of the cooked food were obtained from C.W.A.C. personnel and College students, through the medium of a questionnaire.

The simplest cooking methods were employed. In boiling, the water was always brought to the boiling point, the sprouted seeds added, the container covered tightly and its contents brought back to boiling temperature as rapidly as possible. A minimum amount of water was used, but in cooking sprouted broad-beans they had to be almost covered with water to provide uniform cooking throughout. To preserve vitamin C emphasis was placed on preparing dishes which did not entail loss in the cooking water, e.g., soup, stew, baked dishes and fried dishes. The cooking water was saved for use in soup stocks, gravies or sauces.

Chemical Analysis

A. Ascorbic Acid

The determination of reduced ascorbic acid was carried out as follows:

Two 10-g. samples were ground with acid-washed sand and 25 ml. of normal-sulfuric acid containing 2% metaphosphoric acid. The extracts were centrifuged and washed three times with 20-ml. portions of the acid mixture. The combined extracts were made up to 100 ml. and aliquots were titrated against a standard solution of 2–6 dichlorophenol indophenol, as outlined by Burrell and Ebright (2). To analyze bean sprouts duplicate 25-g. samples were weighed out and transferred to a Waring Blendor with 100 ml. of the acid and reduced to a pulp within 8 minutes. The extracts were then treated as described above but the centrifugate and washings were made up to 200 ml. This method permitted the use of larger and more representative samples as each seedling weighed about 5 g.

When turbid or coloured extracts were encountered, making it difficult to detect the end point, 10 ml. of chloroform were well mixed with an aliquot of the extract and the titration completed, according to McHenry and Graham (3). On centrifuging, two layers were formed and the end point could be determined in the chloroform layer.

Total ascorbic acid determinations (reduced plus dehydroascorbic acid) were made on some of the samples, especially cooked products, employing H_2S reduction. The common procedure is to bubble H_2S through the extract for 15 min. and remove the H_2S with a stream of carbon dioxide or nitrogen. At least 2 hours are required to remove the H_2S, and frequently traces remain which reduce the indophenol dye and give results which are

too high.   To completely remove the H_2S in a shorter time Fellenberg (4) heated the extract and Harris and Olliver (5) put the extract under reduced pressure.   The authors found the following procedure, using the apparatus illustrated in Figure 3 to be most satisfactory.
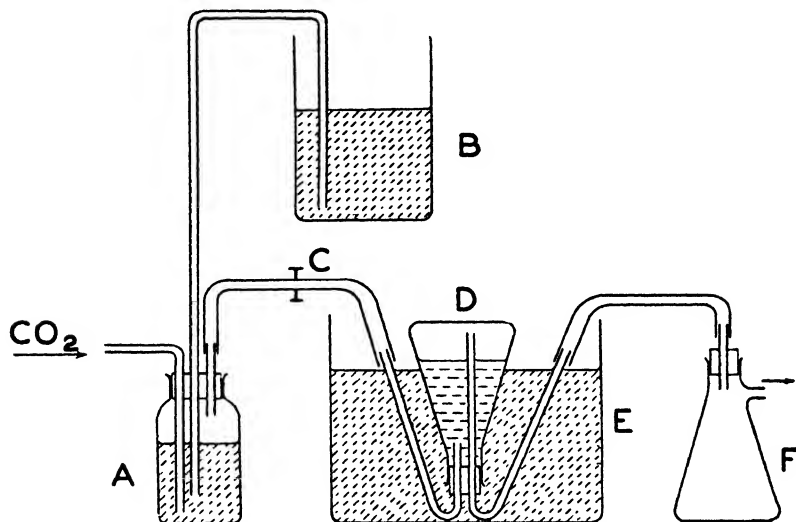

FIGURE 3.   Apparatus for H_2S reduction of dehydroascorbic acid.

A 50-ml. aliquot of the extract, adjusted to pH 3.0, was transferred to a 125-ml. Erlenmeyer flask (D) fitted with a two-hole stopper, an aeration tube with head close to the stopper, and a glass tube extending to the bottom of the flask.   The flask was inverted in a water bath (E) at 55° C., and the H_2S entered through the aeration tube and escaped at the top by the long glass tube.   This device ensured complete saturation of the solution with H_2S and prevented loss due to foaming.   The reduction of dehydroascorbic acid was complete in 15 min. and the extract was then adjusted to pH 2.0 with 50% H_2SO_4.   The flask was replaced in the water bath and connected to a large bottle (A). CO_2 was admitted into A which was filled with water and the gas displaced the water into the elevated bottle (B).   A pinch-cock (C) controlled the gas flow to D and the whole system was put under a reduced pressure of 100 mm. Hg. by applying suction to flask (F).   In this apparatus the extract was freed from H_2S in 15 minutes at 55° C.

It is well known that the indophenol method is affected by the presence of other reducing substances in the extracts.   Roe's colorimetric method (6, 7) employing 2,4 dinitrophenylhydrazine is said to be more specific and also determines dehydroascorbic acid.   These methods were compared with the results shown in Table 1.   In general the results agreed very well and did not indicate, so far as these samples were concerned, that any advantage would accrue from using the more complicated colorimetric procedure in place of the simple titration method.

TABLE 1.—ASCORBIC ACID DETERMINATIONS BY INDOPHENOL TITRATION AND BY THE COLORIMETRIC 2,4 DINITROPHENYLHYDRAZINE METHOD

| | Ascorbic acid—mg. per 100 g. | | | |
| | Reduced | | Total (reduced +dehydro.) | |
	Titration	Colorimetric	Titration	Colorimetric
Broad Beans—sprouted 4 days	24.6	22.9	—	29.2
Vetch—sprouted 3 days	12.1	12.8	—	—
Vetch (cooked 20 min.)	3.5	4.2	—	13.3
Peas—sprouted 10 days	20.5	19.1	21.9	24.5
Dehydrated Cereal Grass	41.2	43.5	—	45.7
Standard Ascorbic Acid Solution (50 mg. per 100 cc.)	50.00	49.8	50.0	50.3

B. Riboflavin

The riboflavin content of the sprouts was determined by the following method which combined certain features of the Chapman and McFarlane (8) and the Najjar (9) fluorimetric methods.

A 10-g. sample was weighed into a 250-ml. centrifuge bottle, 50 to 75 ml. of a 0.2% pepsin solution in 0.33% HCl were added and the mixture incubated overnight at 37° C. and in the dark. After adjusting to pH 4.5, using bromcresol-green as an external indicator, 0.1 g. of takadiastase was added and the incubation continued for 3 to 4 hours. The material was centrifuged, the supernatant transferred to a 100-ml. volumetric flask, the residue was twice washed with distilled water and the combined extracts made up to 100 ml. with distilled water.

5-ml. aliquots were shaken in a separatory funnel with 1 ml. glacial acetic acid and 2 ml. pyridine; 10 drops 4% potassium permanganate were added and after 1 minute 10 drops of 3% hydrogen peroxide. Finally, 5 g. anhydrous sodium sulphate and 10 ml. butyl alcohol were added, the mixture shaken thoroughly for 2 min. and centrifuged. The fluorescence of the pyridine-butyl alcohol phase was measured in a Coleman photofluorimeter. The fluorescence due to substances other than riboflavin was corrected for with a "blank" prepared by exposing the pyridine-butyl alcohol solution to intense ultra-violet radiations, for one hour, thus destroying riboflavin only. The fluorimeter was standardized by carrying out a determination with 5 ml. of a riboflavin solution containing one microgram of pure riboflavin.

EXPERIMENTAL

CEREALS

Cereal seeds are the most desirable from the standpoint of supply and low cost, but oats and barley have their seeds embedded in a coarse hull and are therefore not suitable for sprouting. Hulless oats and barley are now available, but the seed is still expensive and only small quantities are on the market. However, these were studied, along with rye and it was found that, after 3 days of sprouting, the optimum ascorbic acid content was 5–7 mg. per 100 gms. We investigated 5 common wheat varieties:

Red Bog, Thatcher, Apex, Regent and Marquis. When sprouted for 4-days, the ascorbic acid content remained low (1.5–2.5 mg. per 100 gms.) and reached a maximum of 5.5 mg. per 100 gms. at 10 days (Figure 4).
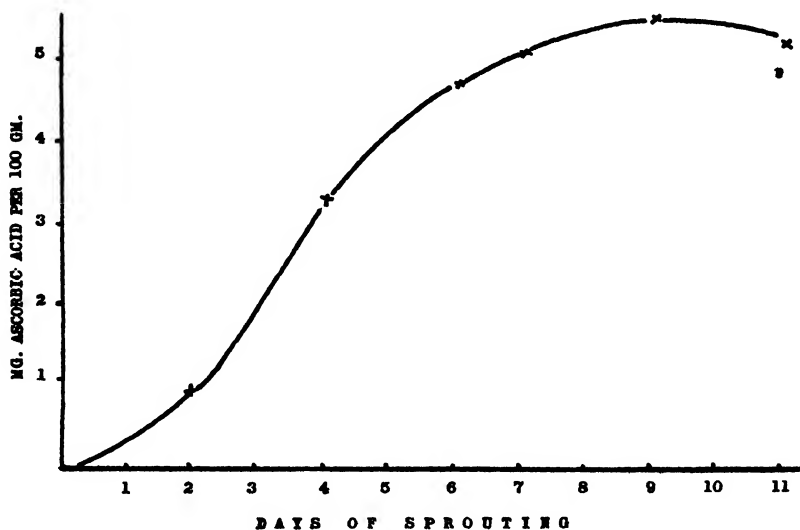
FIGURE 4. Showing the increase in ascorbic acid content of "Regent" wheat, during sprouting.

Cereal sprouts must be considered a poor source of ascorbic acid as 20–30% was lost on cooking for 10 minutes. Cereal sprouts alone were tasteless and had to be blended with oatmeal and served as porridge. It was found that after 3 days sprouting the roots were fibrous and unsuitable for human consumption, so that the sprouts could not be used at the stage of optimum vitamin C content. An attempt was made to remove the fibrous roots but the only practical method was to dry the sprouts, so that the roots became brittle and broke off on subsequent screening; this treatment destroyed the fresh food character of the sprouts.

LEGUMES

Legume seeds do not develop fibrous roots during the early stage of sprouting, and the entire sprout can be eaten up to the stage of the appearance of green leaves. At more advanced stages, only the green shoots are edible. Sprouted Mung beans have been used primarily for dietary purposes but the beans cannot be grown in Canada. Soybean sprouts have attained some popularity, particular emphasis being laid on the protein and ascorbic acid content. The ascorbic acid content of the soybean varieties we have studied was low, amounting to 10–15 mg. per 100 g. after 5 days sprouting. About 30% of the ascorbic acid was concentrated in the tender shoots and was readily leached into the cooking water. The loss after 20 min. cooking was about 50%, thus reducing the ascorbic acid retention to such a low value,that their value was questionable. Since a

great deal of information was already available on sprouting soybeans, we concentrated our efforts on other seeds.

1. Peas

The C. S. I. R. Nutrition Laboratory, University of Adelaide, Australia, recommended the use of Blue Boiler peas, sprouted for 4 days. They claimed a vitamin C content of 38 mg. per 100 g., of which one-third was lost on cooking. This variety is not available in Canada. It was found that, with the variety Ottawa 181, the ascorbic acid content increased to 15–17 mg. per 100 g., after 5 days sprouting. Because of the hardness of the seeds, it was necessary to cook the sprouts for 1 hour to render them soft enough to be palatable, with the result that 80% of the vitamin was destroyed.

To reduce this loss, field peas were sprouted for 10 days, during which time the seeds sent up shoots 8″ long, so that the shoots could be clipped off close to the seeds and served as a green salad. This salad was agreeable to the taste and contained a significant amount of vitamin C. No advantage was found in prolonging growth beyond 10 days, as the shoots became fibrous and the ascorbic acid content declined. The distribution of ascorbic acid between the shoots, cotyledons and roots of pea seedlings at different stages of growth is shown in Figure 5. These sprouts were grown in a constant temperature chamber at 28° C. and 50% relative humidity. The sprouts grew rather poorly because of the low humidity. For comparison, the ascorbic acid content of seedlings from the same seed grown at 90% relative humidity, is also given in Figure 5.
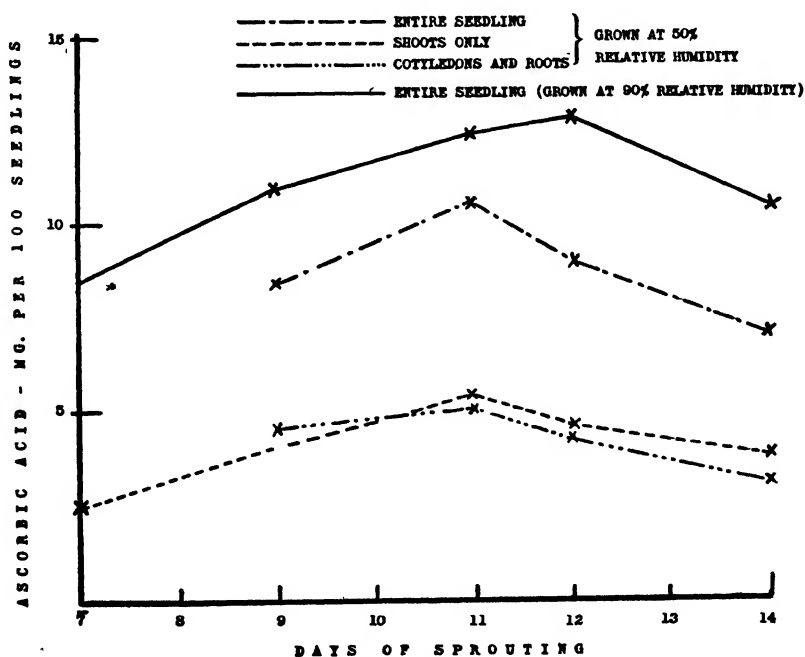
FIGURE 5.   Distribution of ascorbic acid in pea seedlings.

The effect of light on sprouting was next studied, the most apparent response being the attractive green colour and retarded growth; the ascorbic acid content per 100 g. of seedlings was unaffected and an apparent increase in the ascorbic acid content due to light was offset by the lower yield. Ascorbic acid synthesis increased only when the light intensity reached a level which permitted photosynthesis. The effect of light can be seen from the data in Table 2, which also shows that above 25° C. there is a decrease in the yield and ascorbic acid content of the green shoots.

TABLE 2.—THE EFFECT OF TEMPERATURE AND LIGHT ON VITAMIN C SYNTHESIS IN 11-DAY PEA SHOOTS

Treatment	Yield of shoots (gms.)	Ascorbic acid	
		(mg./100 gms.)	(Mg./400 gms. dry seed)
25° C. in light	442	27.0	112
27° C. in light	371	25.9	96
30° C. in light	286	25.9	74
21° C. in dark	515	22.9	118
26° C. in dark	431	22.5	97
30° C. in dark	305	22.2	68

The suitability of sprouting bags (Figure 3) for growing green shoots was investigated. Equal amounts of seed were spread on shallow trays and sprouting bags, and it was found that the yield was greater in the latter case, but the ascorbic acid content of the sprouts was almost identical (Table 3). Rot-proofing the bag prevented the roots from penetrating the material thus facilitating the subsequent cleaning of the bag, although the yield in the presence of the copper carbonate was much lower.

TABLE 3.—THE EFFECT OF DIFFERENT SPROUTING DEVICES ON VITAMIN C SYNTHESIS BY FIELD PEA SPROUTS

Sprouting time (days)	Treatment	Yield of shoots (gm.)	Ascorbic acid content	
			(mg. per 100 gm.)	(mg. per tray)
11	Shallow tray	590	26.6	157
11	Bag, untreated	730	24.5	179
11	Bag, CuCO₃ treated	496	25.2	125

The results in Table 4 indicate the effect of the thickness of seeding the trays. The yield of shoots increased with increasing amounts of soaked seeds per tray. · The thicker the stand the taller the shoots and thus the greater the yield of shoots per 100 g. of soaked seeds. The ascorbic acid content of the shoots increased with the density of the stand but declined when the stand became too thick. The amount of ascorbic acid synthesized per tray was highest where the greatest amount of soaked seed was used, but calculated on a basis of 100 g. of seed, the greatest efficiency was obtained when 1000–1100 g. of peas were spread on one tray.

TABLE 4.—THE EFFECT OF THICKNESS OF SEEDING ON THE YIELD AND ASCORBIC ACID CONTENT OF ELEVEN DAY PEA SPROUTS

Weight of soaked seeds per tray (gm.)	Yield of shoots		Ascorbic acid content		
	(g. per tray)	(g. per 100 gm. soaked seed)	(mg. per 100 gm.)	(mg. per tray)	(mg. per 100 gm. soaked seed)
814	401	49 3	25.7	105	12.9
1014	597	49.0	29.2	174	17.1
1144	606	53.0	30.2	183	15.9
1414	783	55.0	25.5	199	14.0

The riboflavin content of pea sprouts was determined with the results summarized in Table 5. It is evident that these sprouts would not contribute significant amounts of riboflavin to the daily diet.

TABLE 5.—RIBOFLAVIN CONTENT OF FIELD PEAS BEFORE AND AFTER SPROUTING

Sample	Riboflavin (micrograms per 100 gm.)
Dry seed	93
5 day sprouts	131
11 day sprouts	89

Seeds and the sprouts were also treated with growth hormones, including the commercial preparations "Parmone", "Stop Drop", and "Auxilin 1". No beneficial effect resulted from their use at high or low concentrations.

After 10 days sprouting, the pea shoots are crisp and green and ready to serve as salads. These are prepared by clipping the shoots close to the seeds, cutting them into pieces 2–3″ long and "tossing" with French dressing. Attractive and nutritious additions include chopped vegetables, such as tomatoes, radish, carrots, lettuce, cabbage, or sprouted soybeans, which have been boiled 20 minutes and chilled.

The residual seeds would not sprout again although they still contained an appreciable amount of storage nutrients and ascorbic acid. This part of the pea sprout represents a complete loss because of the fibrous consistency of the roots. In 10 days sprouting, 400 g. of dry seed produced about 400 g. of green shoots which contained about 120 mg. of ascorbic acid. Therefore, 100 g. of dry seed produced about 30 mg. of ascorbic acid in the form of edible shoots. An average serving as salad, was 40 g. which provided 12 mg. of ascorbic acid.

2. Navy Beans

The germination of Navy Beans was retarded by soaking; they had to be sprouted between moist towels or on wet sawdust. The seedlings,

sprouted for 5 or 6 days, contained 11–15 mg. ascorbic acid and 125 micro-grams of riboflavin per 100 g. At 12 days a green shoot formed which contained 23–26 mg. ascorbic acid per 100 g. Unlike pea sprouts, the cotyledon of the bean sprouts grew up with the stem to a height of about 4 inches and remained tough and starchy and could not be used in a salad The cooked sprouts contained only 3–4 mg. of ascorbic acid per 100 g. and therefore their use is not recommended.

3. Windsor Broad Beans

The Windsor broad bean (*vicia faba*), sprouted for 4 days, was recom-mended by Johnson and Young (10) for its high vitamin C content. We found that the beans could be sprouted in tubs, trays or bags. Tubs were preferred as the cleanest sprouts were obtained in high yield and with high vitamin C content (Table 6).

TABLE 6.—THE EFFECT OF DIFFERENT SPROUTING DEVICES ON THE VITAMIN C CONTENT OF WINDSOR BROAD BEAN SPROUTS

Container	Weight of edible sprouts*	Ascorbic acid content	
		(mg. per 100 gm.)	(mg. per lot)
	gm.		
Tub	2702	25.5	690
Open bag	2639	28.2	740
Tray	2511	22.8	573

* Produced in 4 days from 2800 g. soaked seed.

The best results were obtained by soaking the seed for 24 hours and watering twice daily (Table 7).

TABLE 7.—THE EFFECT OF WATERING AND SOAKING ON THE 4-DAY SPROUTING OF WINDSOR BROAD BEAN

Soaking time (hrs.)	Daily watering	Weight of edible sprouts from 800 gm. soaked seeds	Number of defective seeds
		gm.	
12	Once	785	22
12	Twice	871	19
24	Once	929	13
24	Twice	977	6

A number of vitamin C determinations were made on several batches of beans sprouted for varying periods of time. The results, presented in Table 8 show that the vitamin C content increased rapidly during the third and fourth day of sprouting. The variability in the results obtained was attributed to changes in temperature during sprouting. Experiments carried out in a constant temperature chamber indicated that the optimum temperature for highest ascorbic acid production was 25° C.

TABLE 8.—SHOWING THE VARIATION IN THE VITAMIN C CONTENT
OF SPROUTED WINDSOR BROAD BEANS

Duration of sprouting (days)	Ascorbic acid—mg. per 100 g.*
2	7.1
3	23.1; 14.6; 23.6; 20 4; 17.5
4	25.6; 15.8; 22.0; 16.9
5	28.3; 22.5; 21.2
7	31.2

* Each value represents the analysis of a different batch of sprouted beans.

The size of the sprouts was related to their ascorbic acid content and the most suitable stage was found to be when the shoots had broken through the seed coat and attained a length of about 1 inch. Extending the sprouting time did not seem beneficial as micro-organisms spread rapidly and caused spoilage and discoloration of the sprouts. Some bacteria caused proteolytic decomposition which produced a disagreeable odour, while others acted on the carbohydrates of the seed, affecting mainly the appearance and, to a lesser extent, the flavour of the sprouted beans. The danger of contamination by pathogenic bacteria does not seem likely.

Riboflavin determinations indicated that Windsor Broad beans, after 2 days sprouting, contained approximately 1 microgram per gram of fresh material.

The cooked sprouts were utilized in many appetizing dishes. The tough seed coats impaired the acceptability of the beans as a vegetable, but they could be removed by blanching which is a tedious procedure but apparently the only practical one. This was done by dropping the sprouted seeds into boiling water and allowing them to stand for 5 minutes, draining and adding cold water. The seeds were allowed to stand for 3 minutes, when the water was again drained off. When cool, the seed coats had loosened and were easily slipped off. The loss of ascorbic acid in blanching amounted to about 10%. Beans with a disagreeable odour were discarded while those with brown spots were considered edible.

Broad beans were served as vegetable, casserol, stew, or as thick soup. In testing for palatability broad beans were served with tomato sauce to a group of C.W.A.C. personnel. Of 33 answers to a questionnaire twenty-eight considered the beans a tasty addition to the diet, while five disliked them. Twenty-five minutes cooking rendered the beans soft to the point of being mushy but a few remained whole and firm. This gave rise to the comment from a few people that the beans were under-cooked. This opinion was based entirely on texture as only a few minutes boiling is necessary to remove the raw flavour from the beans.

When served as a vegetable with green pepper sauce they were generally very well liked. It was suggested that the beans were too dry and broken up. This was overcome by serving with a more generous portion of sauce and by stirring as little as possible during cooking. Comments on the stew were generally favourable.

Vitamin C analyses on the prepared beans showed a loss of 30–50% of the ascorbic acid originally present in the sprouts (Table 9). Further reduction in the final product was due to dilution by ingredients not containing vitamin C. One helping of thick soup contained about 14 mg. of ascorbic acid.

TABLE 9.—THE VITAMIN C CONTENT OF VEGETABLE DISHES OF SPROUTED
WINDSOR BROAD BEANS, AS SERVED FROM AN ARMY KITCHEN

Vegetable dish	Ascorbic acid—mg./100 gm.	
	Before cooking	After cooking
Mixed with tomato	20.5	12.1
Boiled as vegetable	25.5	11.3
Thick soup	20.2	7.6

4. Vetch

Vetch seed (*Vicia sativa*) belongs to the same plant family as Windsor broad beans (*Vicia faba*). The sprouts were equally high in vitamin C, containing about 25 mg. per 100 gm. after 4 days sprouting (Table 10). The seed coat of vetch was not as tough as that of Windsor broad beans, and they could be rendered edible by 20 minutes cooking. The taste of vetch sprouts was not as pleasant as that of Windsor broad beans so they were blended with meat or tomato. One serving of sprouted vetch blended with meat contained 5 mg. ascorbic acid, and when blended with tomato, about 14 mg.

TABLE 10.—THE ASCORBIC ACID CONTENT OF VETCH SPROUTS

Sprouting time (days)	Ascorbic acid (mg. per 100 gm.)	Sprouting time (days)	Ascorbic acid (mg. per 100 gm.)
2	7.3	4	28 2
3	20.5	5	37.4
4	23.4	6	27.5

5. Soybeans

For the reasons already mentioned the work done on sprouting soybeans was very limited. "Mandarin" soybeans (*Glycine Soya*) were soaked for 8 hours at 22° C. and transferred to the sprouting-tubs. A thin, metal lid was placed on top to exclude light and to maintain a high humidity inside. The sprouts were watered frequently, the water being at about 22° C., and stirred by hand to ensure uniform germination. After each watering the tubs drained slowly and completely thus drawing in fresh air. At the end of the sprouting period (4–5 days) the tubs were emptied into a large water tank where the seed-coats floated-off leaving the sprouted seeds ready for use. The data obtained in these experiments is presented in Table 11. · It will be observed that the vitamin C content increased rapidly but failed to reach a high level, and the increase in riboflavin was very slight. The soybeans doubled their weight in sprouting.

TABLE 11.—ANALYSES OF SPROUTED SOYBEANS AT VARIOUS STAGES OF DEVELOPMENT

Sprouting time (days)	Sample	Cooking time (mins.)	Weight of 250 ml. sprouts (gm.)	Weight of 100 seeds (gm.)	Ascorbic acid (mg. per 100 gm.)	Riboflavin (μ g. per 100 gm.)
	Dry seed	—	188.5	17.64	—	—
1	Raw	—	165.5	38.74	1.5	76
	Cooked	60	—	—	1.1	60
2	Raw	—	175.5	40.95	3.2	—
	Cooked	55	—	—	1.8	—
3	Raw	—	146.5	43.29	6.0	—
	Cooked	60	—	—	2.2	—
4	Raw	—	125.5	51.17	10.4	89
	Cooked	40	—	—	1.5	40
5	Raw	—	105.0	66.16	11.4	—
	Cooked	20	—	—	1.7	—

It was interesting to note (Table 11) that the cooking time decreased with prolonged sprouting. This was a great advantage as it saved time and fuel. As a result of the shorter cooking time a higher vitamin C retention was hoped for, but this was not found to be the case. Unfortunately the cotyledon required considerable cooking, consequently the rootlets were overcooked. Thirty per cent of the ascorbic acid in the sprout was concentrated in the tender rootlet and this was readily leached into the cooking water.

Sprouted soybeans were served as a vegetable. The 4-day sprouts were about 2 inches long and the beans were clean and in good condition for serving. Before cooking, the beans were washed thoroughly with large quantities of water and as many of the skins as possible were floated off. The beans were first weighed then dropped into sufficient boiling salted water to cover. They were brought to a rolling boil and allowed to cool in this manner for 40 minutes. As a result of boiling, many of the seed coats came to the top and were skimmed off. At the end of the cooking time the beans were drained in a colander (this drainage water should be used in gravy, white sauce or soup stock) and mixed immediately with a tomato sauce.

Of 33 answers to a questionnaire, 13 liked the soybeans as served while 20 disliked them. The general opinion of the latter was that they were not thoroughly cooked. Our experience was that the beans were cooked from the standpoint of flavour but remained tough from the standpoint of texture. In a further experiment the sprouted beans were boiled for 20 minutes, chilled and served as a salad with chopped carrots, lettuce and mayonnaise. The general opinion was that the salad required too much chewing and the flavour was not well liked. Some, however, appreciated the chewiness, so it seemed that a taste for such a salad might be acquired.

DISCUSSION

The work herein described shows that there are definite limitations in the substitution of sprouts for fresh vegetables. We consider only three types of seed to be of practical importance, viz., field peas, Windsor broad beans, and vetch. Cereal sprouts can only be used in the early stages, i.e. up to 3 days of sprouting. They may then be blended with oatmeal and served as a porridge. Their best feature is their cheapness and abundance, but their nutritional value, so far as ascorbic acid is concerned, is too low to be recommendable.

The attractive appearance and agreeable flavour of pea shoots as a salad met with general approval. Their ascorbic acid content is also high and cooking losses are obviated. The cultivation of pea sprouts, however, takes twice as long as any of the other sprouts. They are the only seed which have to be germinated in thin layers which requires more space and more handling. A tasty and nutritious vegetable soup can be prepared from Windsor broad beans. The ascorbic acid content of the sprouts, originally high, may undergo a considerable loss before serving when cooked too long or not served immediately. The seeds are scarce and expensive. Vetch seeds can be sprouted in a manner similar to that used for Windsor broad beans, but they are not particularly appetizing when served alone. They are palatable when mixed with meat or tomatoes.

The Committee on Food and Nutrition of the American National Research Council (11) has laid down the level for the daily riboflavin requirement at 2.2 to 3.3 mg. for a man weighing 75 kilograms, depending on the degree of physical activity. Considering this as a liberal intake and assuming that 1.8 mg. is sufficient for optimum health, none of the sprouted seeds would contribute a significant amount of riboflavin to the daily diet. The Committee also recommends 75 mg. ascorbic acid daily for the same type of man regardless of his activity. Others (12) believe 30 mg. ascorbic acid is an adequate daily allowance. Recent studies indicate the lower level as being protective against scurvy. With this requirement in mind Windsor broad beans after 5 days' sprouting, common vetch after 4 to 5 days' sprouting and field pea sprouts after 9 to 10 days' sprouting can be recommended as good sources of vitamin C in the diet. One serving of these vegetables will supply one-half the daily requirement of vitamin C. Sprouted soybeans and cereals in the ready-to-serve dish do not contribute appreciable amounts of vitamin C; however, soybeans are valuable for their protein of high biological value and cereals for their vitamin B-complex, so they may be considered a valuable addition to the diet.

Considerable fluctuation was encountered in the vitamin C content of pea sprouts and broad bean sprouts. This was due to variations in the stage of development of the shoot and to changes in external factors during the sprouting period. Reid (13), working with cow pea seedlings, came to the conclusion that the ascorbic acid in the seedlings was partly synthesized from storage carbohydrates and partly through photosynthesis in the young plants. She believed that a simultaneous synthesis and utilization of ascorbic acid took place in the plant and stated that the rate of ascorbic acid breakdown depended on the metabolic rate of the plant. High temperatures increase the metabolic rate, thus explaining the highest vitamin C content in seedlings grown in light at low temperatures.

In our experiment with pea sprouts grown for 11 days, more ascorbic acid per tray was synthesized at 21° C. than at 30° C., while the light of two 100-watt lamps or ultra-violet light had no effect.   The failure to increase ascorbic acid synthesis by light was possibly due to insufficient intensity to permit photosynthesis.   Ultra-violet light does not stimulate photosynthesis and thus was ineffective in producing vitamin C.   Beeskow (14) analyzed Mung beans after 4 days' sprouting and found less ascorbic acid in rapidly growing sprouts than in those which grew more slowly.   No similar observation could be made in this work.   On the contrary, two lots of beans were brought to the same stage of development in 3 and in 5 days respectively.   In both cases the vitamin C content reached 20 to 25 mg. per 100 g.

Cooking methods had a pronounced effect on the vitamin C content of the finished products.   Best results were obtained when the sprouts were dropped into a volume of boiling water just sufficient to cover the sprouts and heated rapidly to bring the water immediately back to the boil. The sprouts were cooked in as short a time as possible and served immeddiately and without draining of the cooking water.   Nevertheless, 35 to 50% of the ascorbic acid was lost in quantity cookery for army purposes.   A further reduction of the vitamin C in the vegetable dish as served was due to dilution with ingredients not containing vitamin C.

SUMMARY

Methods have been developed for sprouting cereal and legume seeds in bulk, and to utilize the sprouted seeds in the diet in the form of palatable dishes.

Wooden tubs were most useful for sprouting Windsor broad beans, vetch, soybeans and cereals, while shallow screen-bottomed trays were best for peas.   An open bag was designed for use in mobile camps and it was found convenient for sprouting all types of seed.   Acceptable dishes were prepared from Windsor broad beans, peas, vetch, soybeans and cereals. The beans were best served as a vegetable, thick soup or in stew; the peas in salad; the vetch in meat or tomato mixtures; soybeans in vegetable dishes or in salads; and cereals in porridge or with rice.

It was estimated that one serving (approximately 6 ounces as vegetable, 2 ounces as salad) of Windsor broad beans, vetch and pea sprouts would supply half the daily requirement of vitamin C.   The amount of riboflavin is considered to be insignificant.

ACKNOWLEDGMENTS

REFERENCES

1. BURKHOLDER, P. R.   Vitamins in dehydrated seeds and sprouts.   Science, 97 : 562. 1943.
2. BURRELL, R. C. and V. R. Ebright.   The vitamin C content of fruits and vegetables. J. Chem. Educ. 17 : 180.   1940.
3. McHENRY, E. W., and M. GRAHAM.   Observation on the estimation of ascorbic acid by titration.   Biochem. J., 29 : 2013.   1935.
4. FELLENBERG, T. V.   Ascorbic acid determination in Swiss foods.   Mitt. Lebensm. Hyg., 32 : 135.   1941.

5. HARRIS, L. J., and M. OLLIVER. The reliability of the method for estimating vitamin C by titration against 2 : 6 dichlorophenolindophenol. Biochem. J. 36 : 155. 1942.

6. ROE, J. H., and C. A. KUETHER. The determination of ascorbic acid in whole blood and urine through the 2,4 dinitrophenylhydrazine derivative of dehydroascorbic acid. J. Biol. Chem. 147 : 399. 1943.

7. ROE, J. H., and M. J. OESTERLING. The determination of dehydroascorbic acid in plant tissues by the 2,4 dinitrophenylhydrazine method. J. Biol. Chem. 152 : 511. 1944.

8. CHAPMAN, R. A., and W. D. McFARLANE. A proposed modification of the fluorimetric method for the estimation of riboflavin. Can. J. of Res. B, 20 : 82. 1942.

9. NAJJAR, V. A. The fluorimetric determination of riboflavin in urine and other biological fluids. J. Biol. Chem., 141 : 355. 1941.

10. JOHNSON, L. P. V., and G. A. YOUNG. The production of vitamin C by sprouting seeds. Natl. Res. Council of Canada, Report No. 5–5–43.

11. Committee on Food and Nutrition, National Research Council of the United States. Recommended daily allowance for specific nutrients. J. Amer. Med. Ass. 116, 2601. 1941.

12. Health Organization of the League of Nations. Report by Technical Commission on Nutrition on work of the Third Session. Bull. Health Organ. League of Nations, 7 : 475. 1938.

13. REID, MARY R. Relation of temperature to the ascorbic acid content of cow pea plants. Bull. Torrey. Bot. Club, 68 : 519. 1941.

14. BEESKOW, H. C. Bean sprouts; their preparation and properties. Michigan Tech. Bull., 184. 1943.

APPENDIX

RECIPES

Sprouted Broad Beans—As a Vegetable

Remove the seed coats from 22 pounds of sprouted Broad beans. Add enough boiling water to almost immerse (ca. 10 quarts), cover tightly with a close fitting lid, bring back to the boil as rapidly as possible and boil 20 minutes. Drain, saving the drainage water for soup stock or sauce, add butter, salt and pepper and serve immediately as a vegetable.

Beans cooked in this manner may be served with many variations of white sauce, e.g., green pepper sauce, cheese sauce, tomato sauce, curry sauce or many other well seasoned sauces.

Broad Bean Casserole

> 20 pounds shelled sprouted broad beans (22 pounds unshelled)
> 4 quarts canned tomatoes
> 2 pounds fat pork
> 8 tablespoons salt
> 8 onions chopped
> 4–6 peppers chopped

Mix all ingredients except the beans, heat thoroughly and pour over the shelled beans. Bake in a moderate oven (350° F.) one hour, or until the beans are soft.

OR

Simmer gently over low heat three-quarters of an hour or until beans are soft, adding water if necessary.

Broad Beans in Stew

In preparing stew for 100 people, 10 to 12 pounds of shelled beans may be substituted for one of the usual vegetables. These are added one-half to three-quarters of an hour before the stew is removed from the heat. Dehydrated vegetables may be used successfully in stew if fresh vegetables are not available.
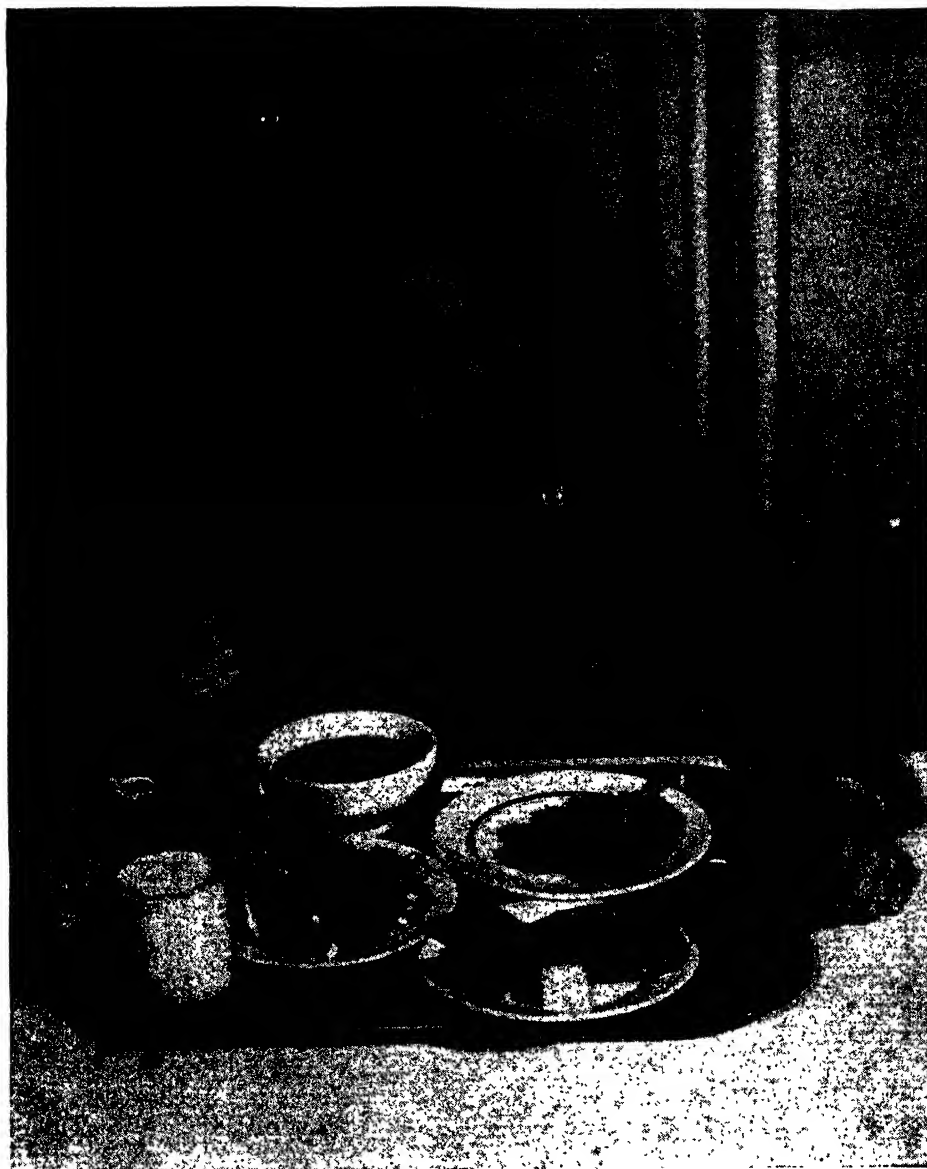
FIGURE 6.   An appetizing and nutritious lunch including sprouted broad bean soup, pea sprout salad and sprouted broad bean casserole.

Broad Bean Soup

    20 pounds shelled sprouted broad beans (22 pounds unshelled)
     6 onions chopped
     2 teaspoons celery salt
     2 tablespoons chile powder (more may be added if desired)
     4 quarts tomatoes
       salt
       pepper

Remove the seed coats from the beans, add chopped onion and enough boiling water (ca. 10 quarts boiling water to 20 pounds of beans) to almost cover.   Boil rapidly one-half hour.   Add tomato and seasoning and heat through, stirring to break up the beans.   If soup is not thick enough, thicken with flour and butter or flour and water.

Chile Con Carne with Sprouted Broad Beans

        10 pounds shelled broad beans
         6⅔ pounds chopped onions
         2⅔ pounds beef suet or other fat
        20 pounds coarsely ground beef
         5⅓ quarts stewed tomatoes
        16 bay leaves
         4 quarts water
         1⅓ cups browned flour
        13⅓ tablespoons chile powder
            salt

Put fat in pan.   Fry onions and beans 10 minutes,.   Add other ingredients and cook about one hour.   Thicken with browned flour. Season to taste.

Fried or Sauteed Sprouted Broad Beans

Fry about 20 pounds shelled beans and eight chopped onions in a small amount of fat about 5 minutes.   Add a small amount of water and let cook 10 to 15 minutes longer.   Serve as a vegetable.

Pea Sprouts—Salad

The 10-day shoots were cut as close to the seeds as possible; cut into convenient lengths (2–3 inches); tossed with French dressing and served. The recipe for the salad dressing was:

        2⅔ tsp. salt
        2 tsp. mustard
        2 tsp. paprika
        1 tsp. pepper
        2 cups vinegar or lemon juice
        4 cups olive oil

The bottom of a wide-mouthed pitcher was rubbed with onion.   All ingredients were put into the pitcher and beaten until creamy with a Dover egg beater and served at once.   This recipe provided enough dressing for 100 salads or more.

Attractive and nutritious additions to this salad were provided by the addition of chopped vegetables such as tomato, radish, carrot, lettuce, cabbage or sprouted soybeans which have been boiled 20 minutes and chilled.

The shoots were also cooked in a minimum amount of water, just enough to prevent burning, and served with butter, salt and pepper as a vegetable like spinach, but this is not recommended as cooking does not improve their quality and destroys a large percentage of the vitamin C.

Sprouted Wheat Porridge

> 1½ cups rolled oats
> 4½ cups water
> 4 cups sprouted wheat
> ¼ tablespoon salt

Cook the rolled oats in the salted water 30–45 minutes; at the same time cook the sprouted wheat 10 minutes in water enough to cover, drain and add to the cooked porridge. This provides enough porridge for 13 people.

In preparing a vegetable from sprouted wheat, fry chopped onion and wheat in a small amount of fat 5–10 minutes. (Overcooking will cause the wheat to become very hard.) Add to this, cooked rice and heat thoroughly in the frying pan. This may be used if the supply of rice is short. When used in soup, drop the sprouted wheat into the soup 15 to 20 minutes before removing from the heat. The wheat has a sweet flavour and requires very little cooking. It may be substituted for rice but requires more chewing

EFFECT OF CERTAIN METHODS OF HANDLING UPON THE BACTERIAL CONTENT OF DIRTY EGGS[1]

C. K. JOHNS[2] AND H. L. BÉRARD[3]

Science Service, Dominion Department of Agriculture, Ottawa

During the summer of 1944, and again to a lesser extent during 1945, an occasional egg broken in plants breaking eggs for the Special Products Board for subsequent drying was found to contain enormous numbers of bacteria while showing no evidence of abnormality in appearance or odour. The organisms isolated were common soil and water types which presumably had gained entrance to the egg subsequent to its being laid (8).

While the studies of Haines (3) indicate that a good quality, fresh, unwashed egg is extraordinarily resistant to bacterial penetration, the consensus of opinion is that dirty eggs, especially if washed prior to storage, are much more likely to become infected (5, 6, 9, 10). Since the washing of dirty eggs on the farm offered a possible explanation for the high count eggs the authors has encountered, arrangements were made with the Poultry Division, Central Experimental Farm, to supply naturally dirty eggs the same day they were gathered. These were then subjected to the treatments outlined below, in the hope that some light would be thrown upon the question.

EXPERIMENTAL

Three times a week, commencing May 16, 1945, one dozen "dirties" were brought to the laboratory. During the first half of these studies, alternate dozens were placed in the 37° C. incubator for 1 hour to warm up before further treatment (to facilitate bacterial penetration of the shell), while the remainder were not warmed up. From each 12 eggs, 6 were left unwashed as controls; the other 6 were washed with a wet cloth, no detergent being used. The cloth was dipped in an enamel measure containing 1500 ml. of water at around 20° C. (68° F.) before wiping the dirt, almost entirely fecal matter, from the shells. Each succeeding egg was washed in the same water, which became progressively dirtier. Without any attempt at drying, the washed eggs were returned to their cartons and, along with the controls, stored at 14° C. (58° F.) with a relative humidity of around 50%. .Three weeks later they were removed; the control eggs were washed in the same way (to minimize contamination of contents during their removal from the shells), and allowed to dry. Each egg was then immersed in a 1/500 solution of Roccal at around 40° C. (104° F.) for a minute or two prior to being opened.

Using sterile forceps, an area of shell large enough to allow the egress of the contents was removed from the blunt end of the egg. The contents were then transferred to a sterile 4 oz. screw cap jar, examined for fluorescence under an EH4 mercury arc lamp equipped with a Corning No. 587 filter (8), and checked for odour and appearance. They were then rendered as homogeneous as possible[4] by means of a mechanical agitator equipped

11

with two sharp blades tilted at about a 30° angle. Plates were poured on appropriate dilutions with tryptone glucose extract milk agar (7) for total count and incubated at 30° C. for 3 days; 1 ml. portions were also plated on violet red bile agar and incubated for 20 to 24 hours at 37° C. Where the bacterial content was high, cultures of the predominant type were isolated for identification and further study.

By the time the first 4 dozen had been analysed, it became evident that very few eggs contained more than an occasional organism. (The highest count noted was 17 per ml.). Since the contamination on the shells of the eggs was almost entirely of a fecal nature, while the organisms isolated from high count eggs have been chiefly soil and water types, it was felt desirable to increase the opportunities for contamination with these types. For this purpose, surface soil was collected from in front of one of the colony houses in use in 1944; this was moistened sufficiently to make a paste, and each egg dipped into it so that approximately 25% of the shell was soiled. The entire dozen eggs were then placed in the 37° C. incubator for one hour, when the washing of one-half the lot was carried out as described.

RESULTS AND DISCUSSION

The distribution of bacteria counts and other relevant data from the variously handled lots of eggs are shown in Table 1.

The most surprising feature is the low percentage of eggs showing any appreciable number of bacteria. Bryant and Sharp (1), examining the whites and yolks of naturally dirtied eggs which had been washed, then held at room temperature for 30 days, found all the way up to 100% containing over 100 organisms per ml.; yolks of unwashed controls were infected in 83% of the cases. (Unfortunately, these workers failed to indicate the magnitude of the counts obtained on infected eggs, so that comparisons on this point cannot be made.) This is the more surprising because our treatments of the eggs, including warming before washing, are believed to be more favourable to bacterial penetration than were the methods they employed.

In our studies, supplementary soiling of the shells with mud resulted in a significant increase in the number of eggs carrying more than 250 bacteria per ml., as well as in the average count per egg. However, the latter figures are greatly influenced by a single egg which gave a count of 62,000,000 per ml.

Washing in itself had much less effect upon the number of infected eggs than had been anticipated. The number of washed eggs with counts in excess of 250 per ml. was identical with that of the unwashed controls (7 in each). However, 4 of the 7 washed eggs gave counts of 2,100,000 per ml. or higher (maximum 62,000,000), while only 1 of the controls showed a count (4,900,000) in excess of 40,000 per ml. (Table 2). The number of bacteria which would be contributed to melange by the washed eggs is therefore significantly higher than for the controls, as indicated by the average counts; 643,778 for 130 washed, and 38,638 for 129 control eggs.

Warming prior to washing also influenced the number of infected eggs less than was expected. In the case of the control eggs which did not

TABLE 1.—DISTRIBUTION OF BACTERIA COUNTS FROM 259 DIRTY EGGS WASHED BEFORE OR AFTER STORAGE FOR 3 WEEKS AT 14° C. (58° F.)

Controls	No. of eggs	Median count	Average count	Count per ml.											
				<1	1-10	11-50	51-250	251-1000	1001-5000	5001-25,000	25,001-100,000	100,001-500,000	500,001-2,500,000	2,500,001-10,000,000	>10,000,000
Washed after storage															
A. Fecal contamination on shell															
1. Not warmed before storage	29	<1	1,380	17	11						1				
2. Warmed before storage	34	<1	1	20	13	1									
B. Feces and mud on shell															
Warmed before storage	66	2	74,913	8	45	3	4	2		2	1			1	
Washed before storage															
A. Fecal contamination on shell															
1. Not warmed before storage	30	<1	7,668	16	12	1						1			
2. Warmed before storage	36	<1	102,786	23	9	1	2	1						1	
B. Feces and mud on shell															
Warmed before storage	64	1	1,255,825	14	40	2	2			2		1			2

TABLE 2.—DATA CONCERNING EGGS SHOWING COUNTS ABOVE 5,000 PER ML.

Egg No.	Bacteria count per ml.	Coliform organisms per ml.	Fluorescence of egg	Predominant organism isolated	Large pores in shell	Treatment of egg					
						Washed before storage	Washed after storage	Warmed before storage	Not warmed before storage	Soiled with feces	Soiled with mud and feces
229	5,600	< 1	—	Micrococcus			x	x			x
368	6,800	< 1	—			x		x			x
245	8,000	< 1	—				x	x			x
424	9,400	< 1	—		x	x		x			x
407	29,000	< 1	—	Escherichia	x		x	x			x
145	40,000	< 1	—	Flavobacterium	x		x		x	x	
186	230,000	< 1	—	Flavobacterium		x			x	x	
302	2,100,000	< 1	+++	Bacterium	x	x		x			x
170	3,700,000	60	—	Flavobacterium		x		x		x	
249	4,900,000	< 1	—	Pseudomonas	x	x	x	x			x
244	15,000,000	< 1	—	Flavobacterium	x	x		x			x
282	62,000,000	< 1	+++	Bacterium	x	x		x			x

receive a supplementary soiling with mud, the warmed eggs actually showed up better than the unwarmed. However, average counts per ml. were significantly higher for the entire lot of warmed eggs, being 441,013 for the latter (200 eggs) and only 4,577 for the 59 unwarmed eggs.

Since eggs with large pores in the shell might be expected to become infected more readily (1, 2), the presence of such pores, as indicated by the escape of air bubbles when the cold egg was immersed in warm Roccal solution, was noted when observed. While few eggs with high counts failed to show large pores, a surprising number with large pores remained virtually free from bacteria.

Examination under ultra-violet light again proved to be of limited value in the detection of high count eggs. As will be seen from Table 2, only 2 eggs showed fluorescence. Both of these gave counts in excess of 2,000,000 per ml. As in our previous studies (8), there was no close correlation between fluorescence in the egg and the presence of organisms of the genus *Pseudomonas*. Fluorescence was not noted in any of the remaining 257 eggs examined.

A word of explanation is necessary concerning Egg No. 407 in which organisms of the genus *Escherichia* were predominant, although no coliform organisms showed up on the violet red bile agar at the initial examination. The tryptone glucose agar plates poured from 1 ml. of this egg showed over 300 mould colonies; one of the moulds isolated was tested out in another connection for its antibiotic potency, and was found to exert a definite inhibitory action on *E. coli*. This may explain the inability of the coliform organisms to develop sufficiently on the violet red bile medium to be recognized after 20 to 24 hours incubation.

In view of the fact that all eggs as received were found to be smeared with fecal matter, it was surprising to find coliform organisms present in only two other eggs. One of these had been warmed and washed before

storage; it gave a count of 60 coliforms per ml. along with a total count of 3,700,000 per ml.   The other, not washed until after storage, gave a count of 2 per ml. with a total count of 6 per ml.

In view of the apparently greater resistance to infection shown by the eggs in these studies, it was felt that some other factor might be involved. Since the eggs studied all came from the Central Experimental Farm flock where the hens receive a ration which is regarded as nutritionally adequate, it seemed possible that this might be a factor inasmuch as many farm flocks may not receive as adequate a ration.   This point may warrant further investigation.

SUMMARY

A surprisingly small percentage of 259 new-laid dirty eggs contained appreciable numbers of bacteria after being stored at 14° C. (58° F.) for 3 weeks.

Washing with a wet cloth before storage did not increase the number of infected eggs, but the average count of such eggs was appreciably higher than that of eggs washed shortly before analysis.

Warming the egg prior to washing also influenced the number of infected eggs less than was expected, although the average count was again higher than for the unwarmed eggs.

Supplementary soiling with mud of eggs naturally soiled with fecal matter increased the number of infected eggs as well as the average count. Coliform organisms were rarely encountered.

ACKNOWLEDGMENTS

REFERENCES

1. BRYANT, R. L. and P. F. SHARP. Effect of washing on the keeping quality of hens' eggs.   J. Agric. Res. 48 : 67-89.   1934.
2. GORESLINE, H. E. "Eggs and Microbiology," in Eggs and Egg Products.   U.S. Dept. Agr. Circ. 583, 1941.
3. HAINES, R. B. Microbiology in the preservation of the hen's egg.   Spec. Rept. No. 47, Food Investigation Board, Low Temperature Research Station, Cambridge, Eng., 1939.
4. HAINES, R. B. The preservation of eggs.   Chem. & Ind. 59 : 391-396, 1940.
5. JENKINS, M. K., J. S. HEPBURN, C. SWAN and C. M. SHERWOOD. Effects of cold storage on shell egg.   Ice & Refrig. 58 : 140-147, 1920.
6. JENKINS, M. K. and M. E. PENNINGTON. Commercial preservation of eggs by cold storage.   U.S. Dept. Agr. Bull. 775, 1919.
7. JOHNS, C. K. Microbiological aspects of egg powder.   Sci. Agr. 24 : 373-382.   1944.
8. JOHNS, C. K. and H. L. BERARD. Further bacteriological studies relating to egg drying.   Sci. Agr. 25 : 551-565, 1945.
9. MALLMANN, W. L. and J. A. DAVIDSON. Oil-protected shell eggs.   U.S. Egg & Poult. Mag. 50 (3) 113-115, 133; (4) 169-171, 189-190, 1944.
10. PENNINGTON, M. E. Bacteria in eggs, in Chemistry & Technology of Food & Food Products, edited by Morris B. Jacobs, Vol. 1, p. 543, Interscience Publishers, New York, N.Y., 1944.

THE EFFECT OF FREEZING AND COLD STORAGE UPON THE BACTERIAL CONTENT OF EGG MELANGE

C. K. Johns and H. L. Bérard

Science Service, Ottawa, Canada

THE EFFECT OF FREEZING AND COLD STORAGE UPON THE BACTERIAL CONTENT OF EGG MELANGE[1]

C. K. JOHNS[2] AND H. L. BÉRARD[3]

Science Service, Ottawa, Canada

The effect of freezing and subsequent storage upon the bacterial content of egg melange has been studied by various workers. Stiles and Bates (16) reported a definite increase in count during the early part of the storage period; melange from strictly fresh eggs increased in count up to 3 months, then declined. Swenson and James (17) showed a drop in count from 150,000 to 60,000 per gram following freezing in dry ice and storage for 8 months at −20° C. (−4° F.). Nielsen and Garnatz (13) reported sharp reductions in counts of whole eggs containing 14% added salt after 41 days storage at −18° C. (−0.4° F.); yolks with 10% added sugar, on the other hand, showed an increase in the 20° C. count after 36 days, with a subsequent decline on further storage. Holtman (3) reported a 99% reduction in total numbers of bacteria and in most instances absence of coliform types after 7 to 9 months storage at −5° to 0° C. (23° to 32° F.) Schneiter, Bartram and Lepper (15) generally found a decrease in count on resampling after 60 hours in the freezer, but in one pack the count increased from 10,000 to 62,000 per gram. Lepper, Bartram and Hillig (11) also found an occasional sample with a higher count after freezing, although the count generally declined.

From the above review, it is difficult to decide just what effect freezing may be expected to have upon the bacterial content of whole eggs. The studies reported in this paper were initiated in the hope of providing a more definite answer.

EXPERIMENTAL

In the first series, sets of 4 samples of melange from freshly filled oblong metal moulds, lined with wax paper, were taken at intervals at a local breaking plant. The moulds were placed in a sharp freezer at approximately −19° C. (−2° F.); 2 days later the frozen blocks (6″ × 7″ × 24″) were bored with a sterilized 1″ auger at the center, near the end, and midway between the first two. A composite sample from the three borings was taken for analysis. Additional samples were taken from the same block after 1, 3 and 6 months storage at approximately −15° C. (5° F.). All samples were brought to the laboratory and analyzed with a minimum of delay. Except where specified, the methods used in official control of Canadian whole egg powder (6) were employed.

In the second series, 20 samples of melange were taken from freshly filled moulds of the same type and size at an Eastern Ontario breaking room, and analyzed at once in the plant laboratory. After 44 hours in a sharp freezer at −21° C. (−5° F.), the frozen blocks[4] were bored as described for the first series, and the samples analyzed without delay.

Since it is well known that many of the organisms in egg melange fail to grow well at 37° C. (7, 13, 15), plates were incubated at 30° C. for 3 days. To obtain some indication of the comparative counts, additional sets of plates from the fresh melange, and from the frozen melange 48 hours later, were incubated at 37° C. for 2 days.

Smears for direct microscopic counts were prepared from the 1:10 dilution of melange, 0.01 ml. being spread over a circular area of 1 cm.^{2}. Those from Series I were stained for 15 to 30 seconds with Gray's (2) stain diluted with 2 parts of water, those from Series II with North's stain (8).

Coliform organisms were estimated using brilliant green bile broth (6), and positive tubes showing black and metallic colonies were considered as containing *Escherichia coli*.

To obtain some idea of the effect of freezing on the flora of the melange, from 70 to 84 colonies were picked from entire plates or segments of plates poured for each of the 4 fresh samples in Lot C (June 7) Series I. This was repeated when re-sampling after storage for 6 months. Cultures were purified and, on the basis of macroscopic and microscopic appearance, supplemented by physiological characteristics, placed in their respective genera.

RESULTS

For Series I plate counts for individual samples are shown in Table 1, and direct microscopic counts in Table 2. The general effect can more readily be grasped from Figure 1, where the average values for all samples in Lots B to F are shown graphically.
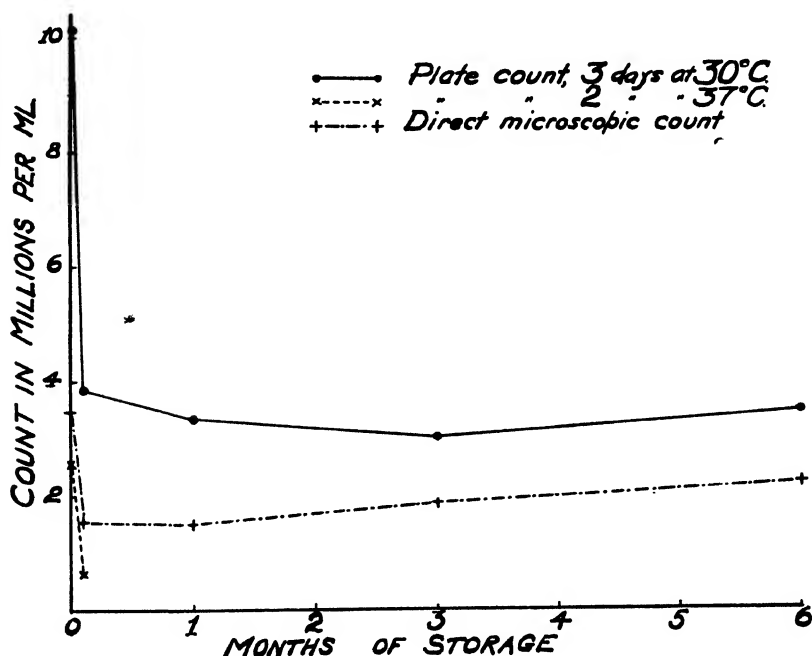
FIGURE 1.—Effect of freezing and subsequent storage on bacterial content of egg melange. (Average of 20 samples).

The results of coliform and *E. coli* determinations for Lot C are shown in Table 3. For Lot A, the dilutions employed were too high, and for the remainder too low, to permit satisfactory calculation of the most probable numbers present, hence data for these lots have been omitted.

The results of the studies on flora of fresh and frozen melange are summarized in Table 4.

For Series II, plate and microscopic counts on fresh and frozen melange are shown in Table 5, together with the percentage survival for each individual sample.

DISCUSSION

The results from Series I show an average reduction in the 30° C. plate count of nearly two-thirds as a result of freezing. On subsequent storage for periods up to 6 months, further changes were slight. The reduction on individual samples in this series ranged from 13 to 87%, with an average of 64.2%. In Series II (Table 5) the average reduction was of the same magnitude (61.66%) but there was less variation between individual samples, values for which ranged from 41.5 to 73.3% reduction. These results are in contrast to those of Holtman (3), who found a 99% reduction in total count after 7 to 9 months storage in a frozen condition (−5° to 0° C. (23° to 32° F.)). This greater reduction may be due to the higher storage temperature; Jensen (5) reported "destruction of bacteria in frozen-egg magma . . . . . . is most rapid when the products are stored at −6.7 to −3.9° C. (20° to 25° F.)."

That a similar reduction in count takes place when melange of lower initial bacterial content is frozen is indicated by data recently furnished by Mr. E. W. Noton, resident inspector for the Special Products Board at Winnipeg. Cartons containing 7 oz. of freshly prepared melange were held in a freezer at −15° to −6° F. (−26° to −21° C.) from June 12 to June 27, 1945, when a chip of frozen melange was removed from each carton, defrosted and analyzed. The remaining contents were then defrosted at room temperature (74° F.), taking 5 hours to defrost completely. After thorough stirring with a sterilized spoon, a second analysis was conducted. Bacteria counts were estimated by means of the Burri slant technique (9), slants being incubated at room temperature for 3 days. The results (Table 6) indicate a reduction in count comparable to that obtained with high count melange by the plate count method (Table 1). The data in the final column, representing analyses of the entire contents of the cartons on defrosting, suggest that some growth took place during defrosting of the remaining contents.

As anticipated, the counts at 37° for 2 days were much lower than those at 30° for 3 days. The average initial 37° count was 25.4% of the 30° count, while that after 48 hours freezing was 21.9% of the 30° count at that time.

As might be expected from the irregular distribution of bacteria in melange (7) the microscopic counts (Table 2) showed considerably more variability than did the plate counts (Table 1). Surprisingly, the microscopic counts were, with rare exceptions, considerably lower than the 30° plate counts, although generally higher than the 37° counts (Figure 1).

Lower microscopic counts than 30° plate counts were also encountered in Series II (Table 5), although the percentage surviving freezing was much higher than that indicated by the plate count.   While a few of the samples reported on by Lepper, Bartram and Hillig (11) showed lower microscopic counts, in most instances the plate counts after 72 hours at 32° C. were lower both before and after freezing.   Our results with fresh and frozen melange are in sharp contrast to those obtained with whole egg powder (6, 8, 11) using North's stain and incubation of plates at 37° C. for 48 hours.   (With powder, plate counts at 30° in 1943 were comparable to those at 37°, although significantly higher 30° counts were obtained in some recent studies (7)).   These results suggest either that certain bacteria in melange fail to stain, or else that some are lost from the smear during defatting, fixing, staining and washing.   The irregular distribution of bacteria in melange, previously mentioned, scarcely affords an adequate explanation of the generally lower level of microscopic counts.

Freezing appeared to reduce the coliform content of melange in much the same way as it did the total count (Table 3).   The *E. coli* content, on the other hand, appeared to be more variable and did not show much drop until after 3 months.   The reduction in coliforms is, however, much less marked than that reported by Holtman (3).

The studies on the effect of freezing upon the bacterial flora yielded rather inconclusive results (Table 4).   Some surprisingly large differences were noted between the various samples in a given lot.   There is some indication that freezing may reduce the proportion of *Pseudomonas* species, a definite drop being noted for each of the four samples analysed.   This is in agreement with the findings of Lochhead and Jones (12) that organisms in frozen-pack vegetables developing at 4° C. (39.1° F.) were least resistant to freezing.   In our studies the number of cultures was too small to warrant the drawing of very definite conclusions.

It was thought that freezing might result in uneven distribution of the organisms within the frozen block.   If ice crystals form first at the periphery, the egg solids and accompanying bacteria might be expected to become concentrated toward the center (1, 14).   To check on this possibility, borings were made in the center, near the end of the block, and midway between the first two borings.   Separate portions were obtained for each boring from the top 2 inches, 2 to 4 inches, and 4 to 6 inches deep.   Two blocks, one from Lot D and one from Lot E, were sampled in this manner after 6 months' storage.   In addition to plate counts at 30° C., total solids were determined.   Contrary to expectations, the highest counts and total solids were found in the top 2 inches, while counts and solids from the center core were lower than those from the end of the block (Table 7).   Somewhat similar counts were obtained by Holtman (4).

It will be observed that in both series the level of counts is far above that ordinarily encountered in melange prepared from good quality eggs. Lepper, Bartram and Hillig (11) state, "In no instance did dried eggs show a microscopic count exceeding 10 millions per gram or frozen eggs 5 millions per gram when they were prepared from sound raw material.   In all cases where these counts were exceeded, decomposed or rotten eggs had been

incorporated in the product or the eggs had been subjected to conditions after breaking-out which permitted them to sour." In our studies we have good reason to believe that neither of the above-mentioned conditions was responsible for the high level of counts. Only graded eggs, preponderantly Grade A, were used. They had been in storage for from several weeks to 3 months; all were examined for odour and appearance on breaking and any of doubtful quality rejected. Plant sanitation was generally satisfactory in both plants, regular check-ups being made by the resident inspectors and plant laboratories, supplemented by occasional sanitation surveys conducted by the senior author. In no instance were conditions or practices encountered which could conceivably result in counts of the magnitude recorded. Studies reported in another paper (10) suggest that high counts on eggs throughout Canada during the summer of 1944 were attributable to a small percentage of apparently normal eggs which contained very large numbers of bacteria. These bacteria were able to penetrate the intact shell of new-laid eggs and to grow to enormous numbers without causing sufficient change in either odour or appearance to warrant their rejection by the breakers.

SUMMARY

The freezing process brought about a sharp reduction in the numbers of bacteria present in whole egg melange. In two series of tests, involving 44 samples, the average reduction in count was nearly two-thirds. Subsequent storage at 5° F. (−15° C.) for 6 months resulted in little further change in count, although *Escherichia coli* appeared to die off after 3 months.

Plate counts at 37° C. for 2 days were less than a quarter of those at 30° C. for 3 days. Direct microscopic counts were almost always lower than plate counts at 30° C. No explanation for this anomaly has been found.

Freezing and subsequent storage for 6 months appeared to reduce the proportion of *Pseudomonas*, but other genera showed variable results.

There was no indication that the freezing process led to a concentration of bacteria and egg solids in the center of the frozen block.

REFERENCES

1. BROWNLEE, B. S. and L. H. JAMES. Bacterial contamination of frozen whole eggs and improved methods of defrosting. Proc. World's Poultry Congr. 1939. Pp. 488-492.

2. GRAY, P. H. H. Two-stain method for direct bacteria count. J. Milk Tech. 6 : 76. 1943.

3. HOLTMAN, D. F. The bacteria of fresh and frozen eggs in relation to public health. (Abstract). J. Bact. 45 : 50. 1943.

4. HOLTMAN, D. F. A bacteriological study of Tennessee market eggs prepared for quick freezing. Mimeographed bulletin, 1944.

5. JENSEN, L. B. Bacteriology of ice. Food Res. 8 : 265-272. 1943.

6. JOHNS, C. K. Microbiological aspects of egg powder. Sci. Agr. 24 : 373-382. 1944.

7. JOHNS, C. K. Unpublished data. 1944.

8. JOHNS, C. K. and H. L. BÉRARD. Direct microscopic method in bacteriological control of dried whole eggs. Food Res. 9 : 396-399. 1944.

9. JOHNS, C. K.   The Burri slant technique in the food industries.   Food in Canada. 4 (3) 17.   1944.

10. JOHNS, C. K. and H. L. BÉRARD.   Further bacteriological studies relating to egg drying.   Sci. Agr. 25 : 551-565.   1945.

11. LEPPER, H. A., M. T. BARTRAM, and F. HILLIG.   Detection of decomposition in liquid, frozen and dried eggs.   J. A.O.A.C. 27 : 204-223.   1944.

12. LOCHHEAD, A. G. and A. H. JONES.   Types of bacteria surviving in frozen-pack vegetables.   Food Res. 3 : 299-306.   1938.

13. NIELSEN, F. A. and G. F. GARNATZ.   Observations on death rates for bacterial flora of frozen egg products.   Proc. 1st Food Conf. Inst. Food Technol. 289-294.   1940.

14. PENNINGTON, M. E. p. 557 of Vol. I, The Chemistry and Technology of Food and Food Products.   Ed. by Morris B. Jacobs, New York.   Interscience Publishers, Inc.   1944.

15. SCHNEITER, R., M. T. BARTRAM, and H. A. LEPPER.   Bacteriological and physical changes occurring in frozen eggs.   J. A.O.A.C. 26 : 172-182.   1943.

16. STILES, G. W. and C. BATES.   A bacteriological study of shell, frozen and desiccated eggs.   U.S. Dept. Agr. Bull. No. 158.   1912.

17. SWENSON, T. L. and L. H. JAMES.   A comparison between eggs frozen at 0° F. and eggs frozen .t −109° F.   U.S. Egg and Poult. Mag. 35 (3) 16-19.   1935.

TABLE 1.—EFFECT OF FREEZING AND SUBSEQUENT STORAGE ON PLATE
COUNTS OF EGG MELANGE.   SERIES I

(Counts in thousands per milliliter)

Sample No.	Prepared for freezing	Before freezing		After 48 hours		After 1 month	After 3 months	After 6 months
		A*	B*	A	B	A	A	A
A 1†	May 8	40	7.7	74	12	—	—	—
2		360	58	180	23	—	—	—
3		750	—	320	—	—	—	—
4		340	—	160	—	-.—	—	—
B 1	June 5	19,000	2,900	4,000	790	3,100	1,000	3,000
2		16,000	3,300	7,300	1,000	6,000	4,400	4,800
3		16,000	3,500	6,600	900	5,900	2,100	4,400
4		15,000	2,500	7,900	1,300	5,500	2,100	5,700
C 1	June 7	7,000	2,100	2,300	530	1,600	2,000	2,300
2		6,900	2,000	1,900	390	1,800	2,500	2,600
3		10,000	2,500	2,500	670	3,300	2,900	2,200
4	⁂	12,000	3,800	2,600	500	2,900	3,200	2,900
D 1	July 3	5,000	960	1,100	310	820	290	620
2		6,900	890	1,100	290	840	290	730
3		4,000	960	1,100	430	610	290	880
4		3,400	650	1,100	420	780	520	690
E 1	July 10	10,000	4,100	4,000	740	3,000	3,600	4,500
2		10,000	2,200	4,900	670	4,700	6,900	6,400
3		5,300	1,100	690	150	990	930	1,500
4		16,000	8,000	4,200	710	2,400	4,500	6,400
F 1	Aug. 2	9,900	—	8,600	—	7,900	8,400	8,200
2		9,700	—	6,600	—	6,600	6,300	6,500
3		13,000	—	6,600	—	5,300	3,200	4,400
4		8,100	—	1,400	—	3,500	2,100	1,600

* A = Incubation at 30° C. for 3 days: B = at 37° C. for 2 days.
† This mould was erroneously placed in a warmer room than the other 3, and except for a crust on the surface was still liquid when sampled after 48 hours.

TABLE 2.—EFFECT OF FREEZING AND SUBSEQUENT STORAGE ON DIRECT MICROSCOPIC COUNTS OF EGG MELANGE. SERIES I

(Counts in thousands per milliliter)

Sample No.	Prepared for freezing	Before freezing	After 48 hours	After 1 month	After 3 months	After 6 months
A 1	May 8	<70	<70	—	—	—
2		<70	<70	—	—	—
3		150	<70	—	—	—
4		150	<70	—	—	—
B 1	June 5	7,300	510	880	340	2,300
2		6,300	1,500	1,500	660	2,600
3		6,000	1,700	1,800	660	1,800
4		5,900	1,200	810	880	2,000
C 1	June 7	730	510	880	590	1,400
2		1,700	1,100	590	660	590
3		1,600	1,200	810	590	370
4		2,200	810	590	940	1,500
D 1	July 3	1,800	1,800	340	370	150
2		1,800	1,500	340	290	370
3		1,500	880	290	370	660
4		3,700	2,500	340	730	810
E 1	July 10	2,100	3,100	1,100	5,900	3,400
2		1,500	3,800	2,100	6,100	8,900
3		940	730	590	2,100	1,800
4		3,200	2,000	1,200	3,700	3,300
F 1	Aug. 2	5,100	3,700	5,000	4,500	2,800
2		5,700	3,200	5,800	4,600	5,800
3		5,200	2,200	2,800	1,600	2,100
4		5,800	1,200	2,400	1,800	1,500

TABLE 3.—EFFECT OF FREEZING AND SUBSEQUENT STORAGE ON THE COLIFORM AND E. coli CONTENT OF EGG MELANGE. (SERIES I)

(Counts as most probable numbers per 100 ml.)

Sample No.	Before freezing		After 48 hours		After 1 month		After 3 months		After 6 months	
	Coliform	E. coli	Coliform	E. coli	Coliform	E. coli	Coliform	E. coli	Coliform	E. coli
C 1	920	79	240	45	350	20	350	110	240	0
2	240	45	240	45	350	41	240	79	350	18
3	1600	69	350	79	240	45	350	79	350	0
4	920	45	350	79	350	20	240	0	240	0

TABLE 4.—TYPES OF ORGANISMS ISOLATED FROM FRESH AND
FROZEN MELANGE. LOT C, SERIES I

Genus	Fresh melange					Melange frozen 6 months				
	C 1	C 2	C 3	C 4	Average for lot	C 1	C 2	C 3	C 4	Average for lot
	%	%	%	%	%	%	%	%	%	%
Proteus	28.2	31.5	33.8	19.0	27.9	12.7	41 0	45.8	41 8	35.1
Flavobacterium	22.5	13.7	36.3	50.0	31.5	17.3	32.0	35.6	33.0	29.5
Pseudomonas	39.5	23.3	16.2	15.5	23.1	20.0	16 7	8.6	12 7	14.6
Achromobacter	5.6	23.3	2.5	10.7	10.4	29.4	7.7	7.1	12.7	14.2
Bacterium	2.8	5.5	10.0	2 4	5.2	7.0	1 3	—	—	2.0
Serratia	1.4	2.7	—	—	1.0	13.4	1.3	2.9	—	4.3
Bacillus	—	—	—	2.4	0.6	1.4	—	—	—	0.3
Sarcina	—	—	1.3	—	0.3	—	—	—	—	—
No. of cultures	71	73	80	84	—	75	78	70	79	—

TABLE 5.—EFFECT OF FREEZING ON COUNTS OF EGG MELANGE.
SERIES II. (AUG. 23-25, 1944)

(Counts in thousands per milliliter)

Sample No.	Before freezing		After 44 hrs. freezing		Survival	
	Plate	D.M.	Plate	D.M.	Plate	D.M.
					%	%
1	8,400	3,700	2,300	—	27.4	—
2	7,700	8,600	3,900	11,000	50.6	128.0
3	11,000	6,400	3,800	6,500	34.5	101.3
4	9,500	5,700	5,100	5,900	53.7	103.8
5	10,000	4,800	3,100	2,400	31.0	49.0
6	13,000	7,300	7,600	6,300	58.5	86.3
7	11,000	5,400	3,200	5,200	29.1	96.4
8	16,000	8,500	4,900	4,800	30.6	56.4
9	9,900	7,400	2,700	2,000	27.3	27.0
10	8,700	5,600	2,500	2,300	28.7	41.1
11	12,000	5,600	3,200	3,200	26.7	57.2
12	12,000	5,100	4,200	2,300	35.0	45.1
13	20,000	5,600	6,100	5,600	30.5	100.0
14	19,000	4,800	—	—	—	—
15	13,000	6,400	6,200	2,800	47.7	43.7
16	17,000	11,000	7,400	8,400	43.5	76.4
17	20,000	9,500	10,000	7,300	50.0	76.9
18	16,000	7,800	8,500	5,700	53.1	73.1
19	21,000	9,300	8,700	7,800	41.4	83.9
20	25,000	9,500	7,300	7,300	29.2	76.9
Average					38.34	73.47

TABLE 6.—EFFECT OF FREEZING AND SUBSEQUENT STORAGE ON BACTERIAL CONTENT OF MELANGE. BURRI SLANT TECHNIQUE. DATA FURNISHED BY E. W. NOTON

(Counts in thousands per milliliter)

Carton No.	At start	After 15 days frozen	
		Chip defrosted	Entire contents defrosted
1	100	42	80
	110	—	76
2	310	140	230
	320	—	260
3	330	200	230
	340	—	260
4	280	130	200
	290	—	160
5	10	12	14
	8	—	12

TABLE 7.—DISTRIBUTION OF BACTERIA AND SOLIDS IN BLOCKS OF FROZEN MELANGE

(Bacteria counts in thousands per milliliter)

—	End of block		Intermediate	Center of block	
	Count	Solids	Count	Count	Solids
		%			%
D 3					
Top 2″	1,400 ⎫		1,200	670	31.48
2″–4″	1,100 ⎬	30.32	1,800	760	28.42
4″–6″	1,300 ⎭		690	630	27.74
E 3					
Top 2″	2,100 ⎫		1,100	1,100	29.71
2″–4″	1,200 ⎬	28.75	810	920	27.88
4″–6″	840 ⎭		840	880	27.14

DRIED WHOLE EGG POWDER

XVIII. THE KEEPING QUALITY OF ACIDULATED, GAS-PACKED POWDERS OF LOW MOISTURE CONTENT[1]

By Jesse A. Pearce[2], Margaret Reid[2], and W. H. Cook[3]

Abstract

Acidification of liquid egg prior to drying did not improve subsequent storage life, although pH measurements showed that powder from untreated egg became acid more rapidly during storage. Reduction in the moisture content (total volatiles) from 4.7 to 3.0% doubled, and reduction from 4.7 to 1.7% tripled, the storage life of dried whole egg powder as assessed by fluorescence tests. The maximum storage life predicted for the low moisture powder by this test was only 36 wk. at 27° C. and 5 wk. at 38° C. Palatability tests suggested that the product was somewhat less perishable, as a powder of 1.7% moisture was considered fit for use as an egg dish after 64 wk. at 27° C. Gas-packing low moisture powders in an atmosphere of carbon dioxide appeared to be slightly more effective as a means of retaining palatability than packing in an atmosphere of air or nitrogen, but was particularly effective in preventing loss of solubility (assessed by potassium chloride values) during storage.

Introduction

During an investigation of the effect of added substances on the keeping quality of egg powder, it was observed that fluorescence development in powders containing either citric or lactic acid was more rapid than in control powder (3). Contrary indications had been observed elsewhere (4).

Accelerated tests, done in these laboratories, showed that the rate of deterioration of egg powder increased with increase in moisture content; therefore, it was recommended that, to maintain quality during storage and transport, dried egg should have a moisture content of not more than 5% and probably 2% or less (7). It was further noted that reduction to 1.4% had marked preservative action, although some deterioration occurred when powders were held at 37° and 48° C. (6).

A study of the effect on keeping quality of packing in nitrogen, carbon dioxide, *in vacuo*, and in the form of compressed tablets showed that only carbon dioxide had a beneficial effect (8). Continued investigation of the effect of carbon dioxide showed that this method of packing afforded some protection against heat deterioration, particularly on the solubility of the powder (6).

At the request of the Advisory Committee to the United States Army Quartermaster Corps, experiments were undertaken in co-operation with American research organizations to verify the advisability of combining those features believed to result in powders of better keeping quality, e.g.,

---

[2] *Biochemist, Food Investigations.*
[3] *Director, Division of Applied Biology.*

---

acidifying liquid egg prior to drying, drying egg to low moisture content, and packing in carbon dioxide or nitrogen or both. The present paper deals with the storage behaviour of such specially treated powders, both at a relatively high temperature, 38° C. (100° F.), and a temperature likely to be met during ordinary handling of dried egg powders, 27° C. (80° F.).

Methods

The quality tests applied were: the evaluation of palatability by a taste panel of six persons scoring on a basis of 10 to 0, 10 being the equivalent of excellent fresh egg (1); a fluorescence measurement of an extract of defatted egg powder in 10% sodium chloride solution (2) and a measurement of solubility in a 10% potassium chloride solution (5). Measurements of pH were made on samples as reconstituted for cooking. Moisture content was determined as total volatiles by a modification of the standard A.O.A.C. vacuum oven procedure. The technique of this modification has been previously described (5).

Materials

The egg powders used in this investigation were prepared in a Canadian egg drying plant from frozen egg. One portion was dried by current methods, resulting in a product having a moisture and volatile content of 4.7%; another portion was dried to have the lowest moisture and volatiles feasible in a single stage commercial operation (3.0%); some of this powder was subjected to a second, combined cooling and vacuum drying process, which further reduced the moisture and volatile content to 1.7%. A further sample of liquid egg was acidified to a pH of 6.7 before subjection to drying and subsequent redrying to produce a low volatile powder (1.7%). These powders were stored in tin plate containers, the headspace gas being air.

Samples of the acidified and untreated products were also packed in tin plate in atmospheres of carbon dioxide and nitrogen. The carbon dioxide content of the headspace gas was approximately 100, 75, 50, 25, and 0%; the remainder of the gas was nitrogen except for a trace of oxygen.

Powders stored at 38° C. (100° F.) were sampled after 1, 2, 4, 8, and 16 weeks' storage, while powders stored at 27° C. (80° F.) were sampled after 4, 8, 16, 32, and 64 wk.

Results

The effect of moisture content is shown in Fig. 1, while effects of acidifying liquid egg and of using atmospheres of carbon dioxide and nitrogen, either alone or mixed, are given in Tables I and II and Fig. 2. Only the mean values for each variable, averaged over all other conditions, are shown in Tables I and II, since this was a convenient method of summarizing the data. The inert atmosphere in the headspace of the tins is recorded on an "as-packed" basis. Measurement after the one, two, and four weeks at 38° C.

and after four and eight weeks at 27° C. showed that the pressure inside the container had been reduced by about $\frac{1}{3}$ atm. and that the carbon dioxide content of the headspace gas was 90, 45, 5, 2, and 0%, the remainder of the gas being nitrogen and about 0.5% oxygen. Sorption of carbon dioxide by the powder was believed responsible for this phenomenon and is under investigation.

MOISTURE EFFECTS
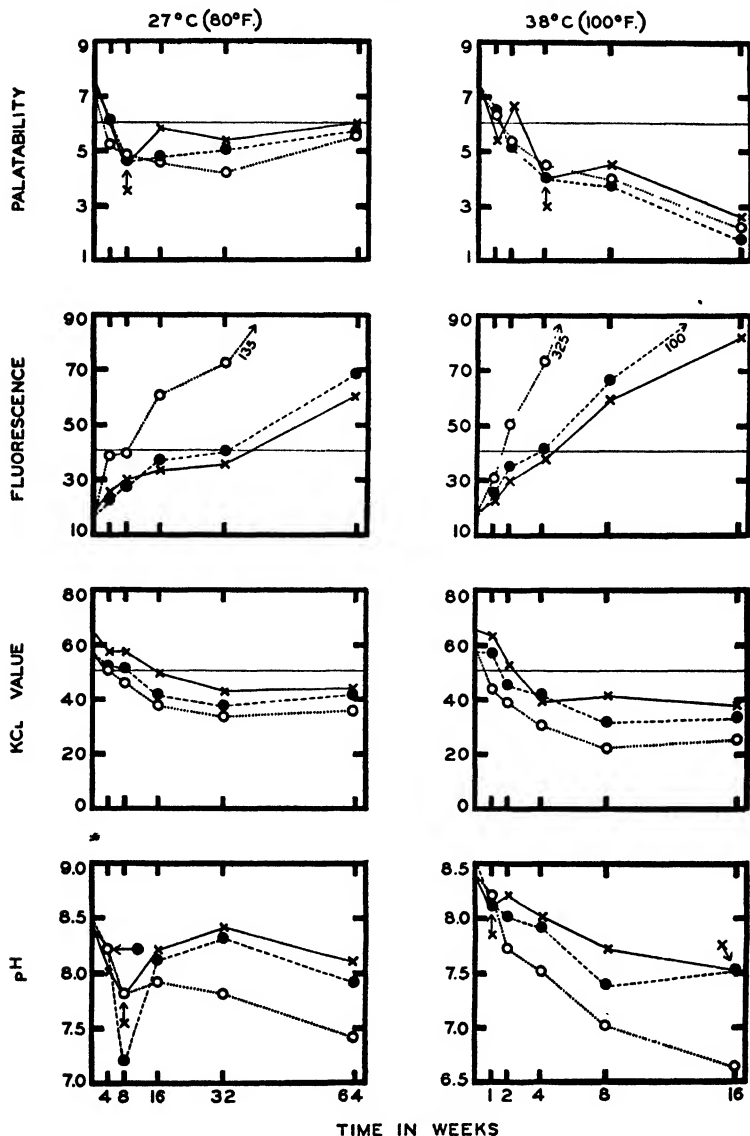
FIG. 1. *The effect of moisture levels on the keeping quality of dried whole egg powder stored at 27° C. (80° F.) and 37° C. (100° F.). Light lines show limits of desirability as an egg dish. 4.7% moisture,* o; *3.0% moisture,* •; *1.7% moisture,* ✕.

TABLE I

TABLES OF MEANS AND ANALYSES OF VARIANCE OF TESTS ON EGG POWDER OF 1.7% MOISTURE
CONTENT PREPARED FROM LIQUID EGG WITH AND WITHOUT ADDED
ACID AND STORED IN ATMOSPHERES OF CARBON DIOXIDE
AND NITROGEN AT 38° C. (100° F.)

Tables of means

Variable under study	Palatability	Fluorescence value	Potassium chloride value	pH
Powder				
From acidified liquid egg	5.6	38.6	55.5	7.3
From untreated liquid egg	5.6	36.5	53.9	8.0
Approximate gas composition[1]				
100% carbon dioxide	5.7	35.2	57.6	7.6
75% carbon dioxide	5.8	35.5	56.4	7.6
50% carbon dioxide	5.6	38.0	53.8	7.7
25% carbon dioxide	5.7	39.0	53.2	7.7
0% carbon dioxide	5.3	40.3	52.4	7.7
Storage time				
Initial	7.7	17.1	63.1	7.9
1 week	6.8	21.1	64.8	7.8
2 weeks	6.2	27.3	61.4	7.8
4 weeks	4.7	33.2	50.7	7.6
8 weeks	4.5	50.1	47.8	7.4
16 weeks	3.6	76.9	40.4	7.6

Analyses of variance

Variance attributable to:	Degrees of freedom	Mean square			
		Palatability	Fluorescence value	Potassium chloride value	pH
Powder (plain vs. acid)	1	0.00	66 **	35 *	7.9 **
Gas composition	4	0.40	58 **	61 **	0.026
Storage time	5	24.2 **	5035 **	968 **	0.452*
Powder × gas composition	4	0.06	5.6*	11	0.022
Powder × storage time	5	0.21	67 **	17 *	0.060*
Storage time × gas composition	20	0.35*	11.4**	7.3	0.026
Residual	20	0.15	2.0	4.8	0.020

[1] *Remainder of gas, nitrogen.*
   * *Exceeds the 5% level of statistical significance.*
   ** *Exceeds the 1% level of statistical significance.*

Acid Powder

Acidifying liquid egg prior to drying appeared to afford little protection to
the powder (Tables I and II). The only measurement showing significant
differences between powders at both storage temperatures was, of course, pH.
At 38° C., in addition to difference in initial pH, the powder prepared from
untreated egg tended to become acid more rapidly than powder prepared from

TABLE II

TABLES OF MEANS AND ANALYSES OF VARIANCE OF TESTS ON EGG POWDER OF 1.7% MOISTURE CONTENT PREPARED FROM LIQUID EGG WITH AND WITHOUT ADDED ACID AND STORED IN ATMOSPHERES OF CARBON DIOXIDE AND NITROGEN AT 27° C. (80° F.)

Tables of means

Variable under study	Palatability	Fluorescence value	Potassium chloride value	pH
Powder				
From acidified liquid egg	6.2	27.6	57.0	7.4
From untreated liquid egg	6.3	28.4	56.8	8.2
Approximate gas composition[1]				
100% carbon dioxide	6.3	27.2	59.3	7.8
75% carbon dioxide	6.4	26.4	58.2	7.8
50% carbon dioxide	5.9	27.7	56.6	7.8
25% carbon dioxide	6.3	27.8	55.6	7.8
0% carbon dioxide	6.0	30.9	54.8	7.8
Storage time				
Initial	7.7	17.1	63.1	7.9
4 weeks	5.4	22.2	60.5	7.8
8 weeks	5.6	23.2	62.4	7.6
16 weeks	5.7	29.5	55.8	7.8
32 weeks	6.5	29.4	51.7	8.1
64 weeks	6.3	46.6	47.9	7.6

Analyses of variance

Variance attributable to:	Degrees of freedom	Mean square			
		Palat-ability	Fluores-cence value	Potassium chloride value	pH
Powder (plain vs. acid)	1	0.00	11	1.01	8.4 **
Gas composition	4	0.45	36 **	41 **	0.26 **
Storage time	5	7.4 **	1057 **	385 **	0.385**
Powder × gas composition	4	0.81	6 7	8 8	0.016
Powder × storage time	5	0.36	1.0	8.4	0.014
Storage time × gas composition	20	0.24	5.4	7.5	0.016*
Residual	20	0.32	3.7	5.3	0.006

[1] *Remainder of gas, nitrogen.*
* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% of statistical significance.*

acidified liquid egg. While it is difficult to explain this phenomenon, it is believed of little practical importance since acidification of liquid egg prior to drying had no other significant effect.

Effect of Moisture Content

There is evidence from Fig. 1 that reduction in moisture content resulted in a slower rate of decrease in palatability during storage. Powder with a moisture content of 1.7% was about one palatability unit better than that

GAS – PACKING EFFECTS
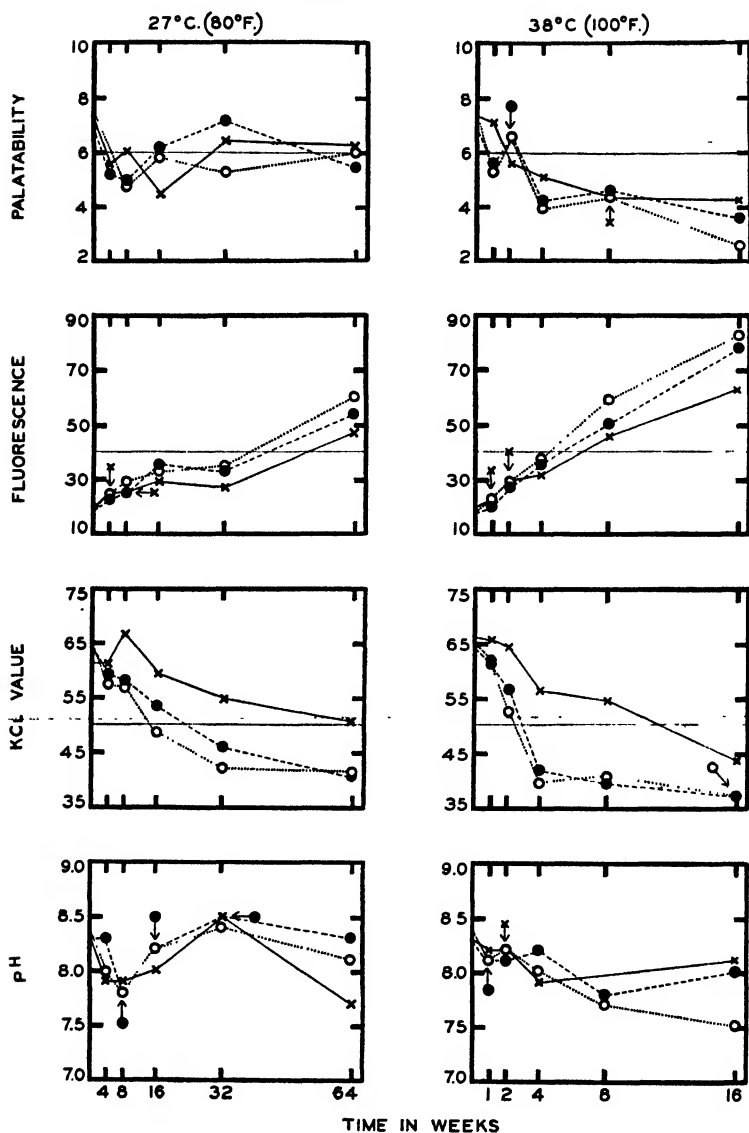
FIG. 2. *The effect of gas-packing on the keeping quality of dried whole egg powder (moisture and volatiles 1.7%) stored at 27° C. (80° F.) and 37° C. (100° F.). Light lines show limits of desirability as an egg dish. Air, o; nitrogen, ●; carbon dioxide, ✕.*

with 4.7% moisture after storage for 16 wk. at 38° C. and about half a palatability unit better after 64 wk. at 27° C. Powders stored at 27° C. for eight weeks had exceptionally low palatability and pH values; nevertheless, the 1.7% moisture powder was still considered suitable as an egg dish after 64 weeks' storage in an atmosphere of air at 27° C.

It had been noted that fluorescence values of 40 are about the equivalent of a palatability score of 6, the limit of desirability of the powder as an egg dish (1). Using this criterion, it was observed that the reduction in moisture to 3%, doubled, while further reduction to 1.7%, tripled the storage life at 38° C. (Fig. 1). However, even this extended life was only five weeks. At 27° C., powders having 4.7% moisture had a storage life of only about six weeks, while powders of 3.0 and 1.7% moisture withstood storage of 32 and 36 wk., respectively.

Measurements of potassium chloride value and pH, both related to palatability (5), also indicated an increase in storage life as volatile content was reduced.

Packing in Carbon Dioxide and Nitrogen

The effects of gas-packing egg powder of 1.7% volatile content in atmospheres of air, carbon dioxide, and nitrogen are shown in Fig. 2. The values depicted here contrast data from Fig. 1 with data from Tables I and II. Only the data for air, nitrogen, and carbon dioxide packing are shown since these showed most clearly the differences between methods of packing. Again palatability scores were variable, but there was some indication of increased storage life as a result of gas-packing, carbon dioxide being more effective than nitrogen, which was in turn more effective than air. Fluorescence and pH measurements also indicated that carbon dioxide was more effective than nitrogen in increasing storage life. The carbon dioxide packed material showed a marked drop in pH after 64 wk. at 27° C., thus explaining the significant differential effect noted in Table II. This may be the result of some reaction between carbon dioxide and egg powder to produce more highly acidic products.

Generally, slight improvement in palatability resulted from increasing the carbon dioxide content in the headspace gas (Tables I and II). Both fluorescence and potassium chloride values indicated that improvement resulted from increased carbon dioxide content. This improvement became more noticeable as storage progressed.

The most pronounced effect was evident in the improved solubility of the powders packed in an atmosphere of carbon dioxide: after eight weeks' storage at 38° C., powder in an atmosphere of carbon dioxide had a potassium chloride value of 55, while powders packed in air or nitrogen had a value of about 38. After 64 wk. at 27° C. the carbon dioxide packed material still had a solubility greater than 50.

Acknowledgments

References

1. PEARCE, J. A. and THISTLE, M. W.   Can. J. Research, D, 20 : 276-282.   1942.
2. PEARCE, J. A., THISTLE, M. W., and REID, M.   Can. J. Research, D, 21 : 341-347.   1943.
3. PEARCE, J. A., WOODCOCK, A. H., and GIBBONS, N. E.   Can. J. Research, F, 22 : 34-38. 1944.
4. STEWART, G. F., BEST, L. R., and LOWE, B.   Proc. Inst. Food Tech. 77-89.   1943.
5. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E.   Can. J. Research, D, 21 : 1-7.   1943.
6. THISTLE, M. W., WHITE, W. H., REID, M., and WOODCOCK, A. H.   Can. J. Research, F, 22 : 80-86.   1944.
7. WHITE, W. H. and THISTLE, M. W.   Can. J. Research, D, 21 : 211-222.   1943.
8. WHITE, W. H., THISTLE, M. W., and REID, M.   Can. J. Research, D, 21 : 271-276.   1943.

DRIED MILK POWDER

IV. THE EFFECT OF STORAGE TEMPERATURE, MOISTURE CONTENT, AND PLANT SOURCE ON THE KEEPING QUALITY OF MILK POWDERS OF DIFFERENT FAT LEVELS[1]

By W. A. Bryce[2] and J. A. Pearce[2]

Abstract

Milk powders with fat contents of 1, 26, 28, and 30% from two plants were tempered to moisture contents of 2, 3, and 5% and stored for periods up to 16 weeks at temperatures of from 40° to 140° F. Appreciable deterioration, assessed by palatability, occurred in the whole milk powders stored at temperatures of 60° F. and higher, and there was considerable difference in the stability of powders from the two plants. For both plants, the keeping quality of powders of 26 and 28% of butter fat was equal. At 80° F. and lower, the powder containing 30% of butter fat was more stable than the 26 and 28% powders from the same plant, but at higher temperatures the 30% powder deteriorated more rapidly. At 80° F. the average decrease in palatability of whole milk powders with 2% moisture was two palatability units. The palatability of the skim-milk powder increased greatly at all temperatures during the early part of the storage period, but later decreased at temperatures of from 100° to 140° F. Skim-milk powder of 2% moisture stored at 80° F. had a palatability score 2.5 units higher than the initial score. In general, a moisture content of 3% was preferable to moisture contents of 2 and 5% for both whole and skim-milk powders. The differences in stability of powders from different plants were enhanced by increased moisture contents and higher storage temperatures.

Introduction

Experience gained during the war years has emphasized that improved methods of handling and storing must be developed if dehydrated foods are to assume their proper place in the post-war period. The importance of dried milk in the national diet has focused considerable attention on studies of production methods and keeping quality of this material.

Studies on the effect of temperature on the development of storage flavours in dried milk have yielded conclusions that have been somewhat conflicting. In one investigation (1) little difference was observed between powders stored at 40° F. and at 68° F., but a marked difference at 100° F. was reported. Powders from partially skimmed milk did not develop tallowiness when stored for 18 months at 32° F., but at 68° F. this off-flavour was noticeable in from five to six months. The rate of deterioration of whole milk powders has been found to increase rapidly at temperatures above 32° F. (4). It has also been reported (9) that the palatability decreased more rapidly at 117° F. than at 100° F. A storage temperature of 100° F. has been found to be better than either 80° or 120° F. (7).

The effect of moisture on the keeping quality of both spray and drum-dried powders has been studied by a number of investigators (1, 2, 4, 9) who have found that moisture contents below 2% accelerated the development of tallowiness in spray-dried powders, and that at moisture contents above 5%, deterioration occurred rapidly in powders of all types. Little difference was observed in the effect of moisture contents between 2.3 and 5.4% on the development of tallowy flavours in powders of 30% butter fat, but with powders of 28% fat, a moisture content of 3.5% was preferable to either 2 or 5%. In general it has been found that the optimum moisture content required to reduce the development of storage flavours to a minimum is between 2 and 3%. It has been recognized that the keeping quality of dried milk is a function of both storage temperature and moisture content, increased moisture contents accelerating the effect of high storage temperatures.

It is evident that some disagreement exists in the published data on the keeping quality of milk powders. This paper discusses an investigation designed to evaluate the effects of moisture content and storage temperature on keeping quality, and at the same time to consider the effects introduced by different processors, and by the use of powders of different fat levels.

Materials and Methods

The powders used were commercial spray-dried products made by two Canadian companies from milk produced in the spring of the year. Powders of 1, 26, and 28% of fat were supplied by one plant, and of 1, 26, 28, and 30% of fat by another.

Each type of milk powder was divided into three portions, one of which was tempered to a moisture content of 2% by vacuum desiccation over phosphorus pentoxide, and was stored in tin plate (air as headspace gas) at temperatures of 40°, 60°, 80°, 100°, 120°, and 140° F. (4.4°, 15.6°, 26.8°, 37.8°, 49°, and 60° C.). The moisture contents of other portions were adjusted to 3 and 5%, respectively, by exposure to an atmosphere of high humidity, and were stored in a manner similar to those at 2% moisture, but at temperatures of 80⁶, 100°, and 120° F. only.

Palatability was the only quality test used that was satisfactory (7). The powders were tested for quality both initially and after storage for 2, 4, 8, and 16 weeks by an organoleptic method previously reported (7). The reconstituted milk was scored by a panel of 14 tasters and given a rating of from 10 to zero, 10 being the equivalent of best quality fresh whole or skim-milk.

Results

Effect of Temperature

The palatability results were analysed by statistical methods. The significant effects of different temperatures on each of the whole milk powders of 2% moisture are shown graphically in Fig. 1, and a summary showing averages for all products stored at the different temperatures is presented in
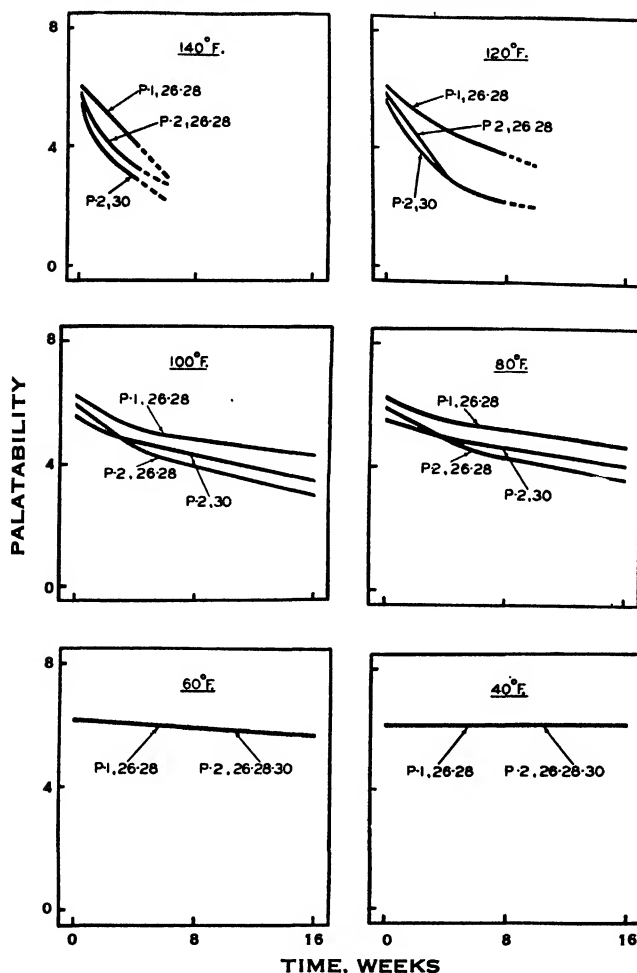
FIG. 1. *The effect of storage temperature on the palatability of whole milk powders of different fat levels from Plants 1 and 2. All samples at 2% moisture.*

Fig. 2. Although there was an appreciable difference between powders from the two plants, the powders of 26 and 28% butter fat from either of the plants behaved in a comparable manner at each storage temperature.

At the higher temperatures of 80° to 140° F., the material from Plant 1 was given a consistently higher palatability score than that of comparable fat content from Plant 2, showing that the product from Plant 2 was less stable than that from Plant 1. At the lower temperatures, the behaviour of all powders was the same. At 80° and 100° F., the 30% butter fat powder was given a higher palatability rating than the 26 and 28% powders from the same plant. At 120° and 140° F. the 30% powder was scored lower than the 26 and 28% powders.

Storage for 16 weeks at temperatures of 60° F. and lower had little effect on the palatability of all whole milk powders investigated, although a slight

downward trend was observed at 60° F.   Typical storage flavours developed in powders at temperatures of 80° F. and higher, the rate of deterioration increasing with temperature.   The data in Fig. 1 do not agree with observations previously reported (6, 7, 10) that milk powder maintained a higher palatability when stored at 100° F. than at either 80° or 120° F.
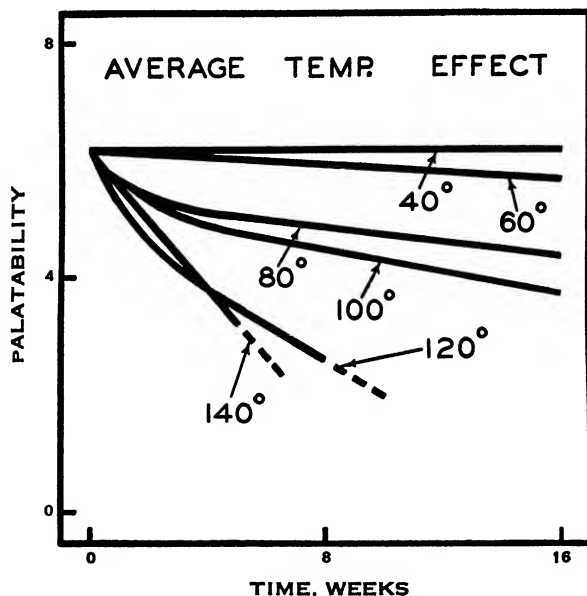
FIG. 2. *The effect of storage temperature on the palatability of whole milk powders (averages of all samples) at 2% moisture.*

The existence of an induction period in the deterioration of milk fat has been reported (5), but the present data gave no indication of such a phenomenon when palatability was used as a quality test.   It can be seen from Fig. 1 that the rate of deterioration of all whole milk powders at all temperatures approximated a straight line relation, although the decrease in the rate of deterioration previously observed (7, 10) was noticeable.   If an induction period existed, it must have come within the first two weeks of storage.

A comparison of the deterioration in skim-milk powder from the two plants is shown graphically in Fig. 3, and a summary of the temperature effects is presented in Fig. 4.   Considerable difference was observed in both the initial palatability and the changes occurring during storage.   The data for 40° and 60° F. have been combined as there was no real difference between the values for the powders at these two temperatures.

In contrast to the results obtained from the study of whole milk powders, it was found that the skim-milk powder from Plant 2 was much superior in keeping quality to that from Plant 1.   However, throughout the experiment, the over-all behaviour of the two skim-milk powders was almost parallel at all temperatures.   Under all conditions both products exhibited an initial

increase in palatability over the first four weeks of storage. At temperatures of 140°, 120°, and 100° F. the trend was downward after this initial increase was past, but below 100° F. there was no appreciable change during the next 12 weeks. The average palatability of both powders rose to a higher value as the temperature decreased (Fig. 4).
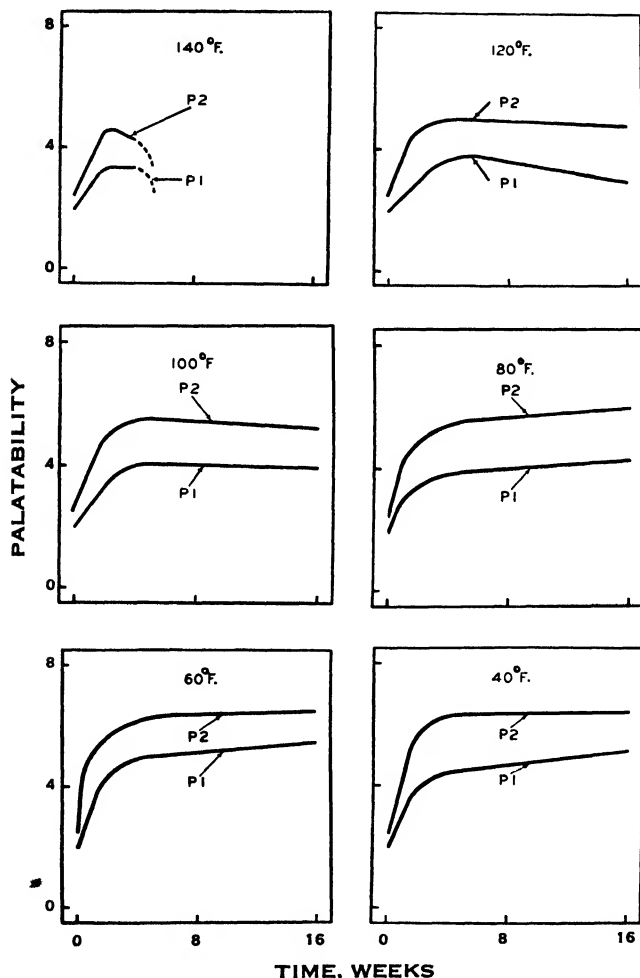
FIG. 3. *The effect of storage temperature on the palatability of skim-milk powders from Plants 1 and 2. All samples at 2% moisture.*

Effect of Moisture

The significant effects of moisture content on the keeping quality of dried whole milk powders are shown in Fig. 5. As in Fig. 1, the data for powders of 26 and 28% butter fat are combined, as the behaviour of these powders was identical. The different behaviour of the 30% butter fat powder was observed at all moisture levels. At 80° F. the 30% powder was given a
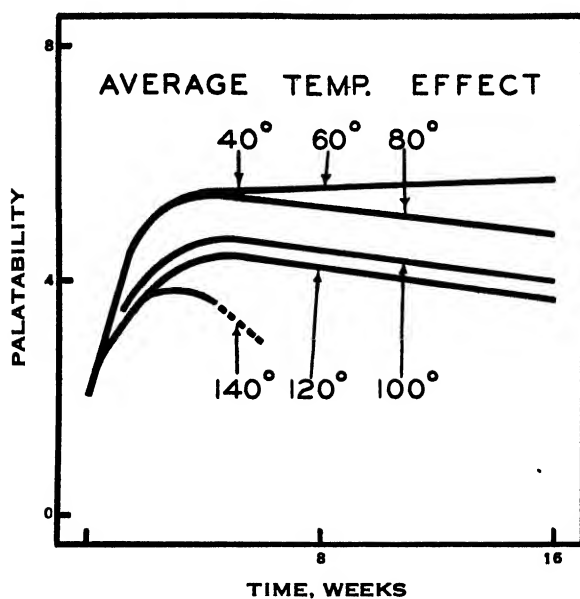
FIG. 4. *The effect of storage temperature on the palatability of skim-milk powders (averages of all samples) at 2% moisture.*
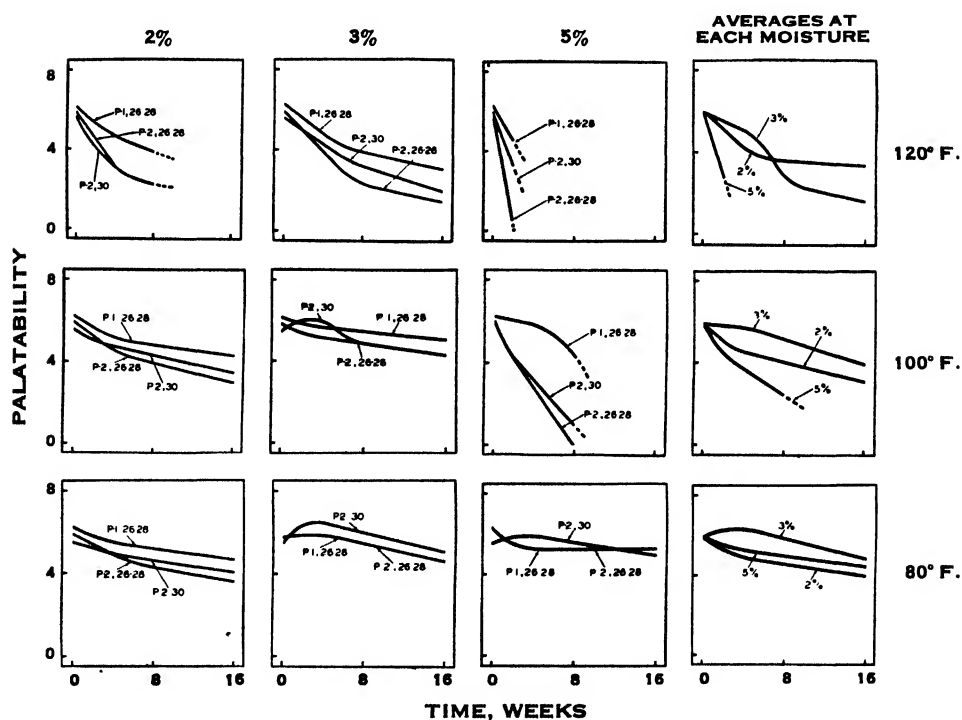
FIG. 5. *The effect of moisture content and storage temperature on the palatability of whole milk powders from Plants 1 and 2.*

slightly higher palatability rating than the 26 and 28% fat powders from the same plant. For all moisture levels, at higher temperatures, the difference in origin of the powders introduced greater variations than did the difference in fat content. The variation in stability of the powder with plant source was enhanced by increased moisture contents and by higher storage temperatures. A comparison of powders of equal fat levels at 100° and 120° F. showed that the keeping quality of powder from Plant 1 was superior to that of the powder from Plant 2 at all moisture levels.

The effect of moisture on the keeping quality of the powders was marked. At 120° F. the palatability of the powders of 2 and 3% moisture decreased at comparable rates, but those at 5% moisture deteriorated very rapidly. Considerable browning of these latter powders was observed after only two weeks of storage, and the palatability had decreased to such an extent that no further tasting was done. At 100° F. the powders with 3% moisture were markedly superior to those with moisture contents of 2 and 5%. At 80° F. the quality of the 3% moisture powders was appreciably better than that of the 2 and 5% powders although the differences were not as great as they were at 100° and 120° F. Only at 80° F. did the quality of the powders of 5% moisture remain higher than that of the powders of 2% moisture.

The combined effect of moisture content and storage temperature on keeping quality of whole milk powder can readily be seen from the graphs in Fig. 5, which show averages for all powders at each moisture level. At temperatures of 80° and 100° F., the superiority of the 3% moisture powder was evident. This was also evident during the first eight weeks of storage at 120°, but by the end of the sixteenth week the palatability of the 3% moisture powder had dropped below that of the 2% powder. Both the rate of deterioration shown by the slope of the curve and the actual palatability level of the powder were dependent on moisture content and storage temperature.

The data for the study of moisture effects on the keeping quality of skim-milk powders are shown graphically in Fig. 6. The superiority of the powder from Plant 2 over that from Plant 1 was observed for all moisture levels. The palatability change in the two products was about the same, the principal difference between them being in the initial quality.

As shown by the graphs of average palatabilities at each moisture level, the powders at a moisture content of 3% were again found to be superior to those at 2 and 5%, although the difference between the 2 and 3% powders was not marked. Storage life of the powder containing 5% moisture was considerably shortened at 120° and 100° F. but at 80° F. this powder was scored as high as were the 2 and 3% powders.

The marked initial increase in palatability exhibited by the skim-milk powder with a 2% moisture content was also observed for the 3 and 5% powders. At 80° F., this increase continued in powders of 2 and 5% moisture throughout the storage period. The level to which the palatability of the 5% powders rose was found to decrease with increasing temperatures.
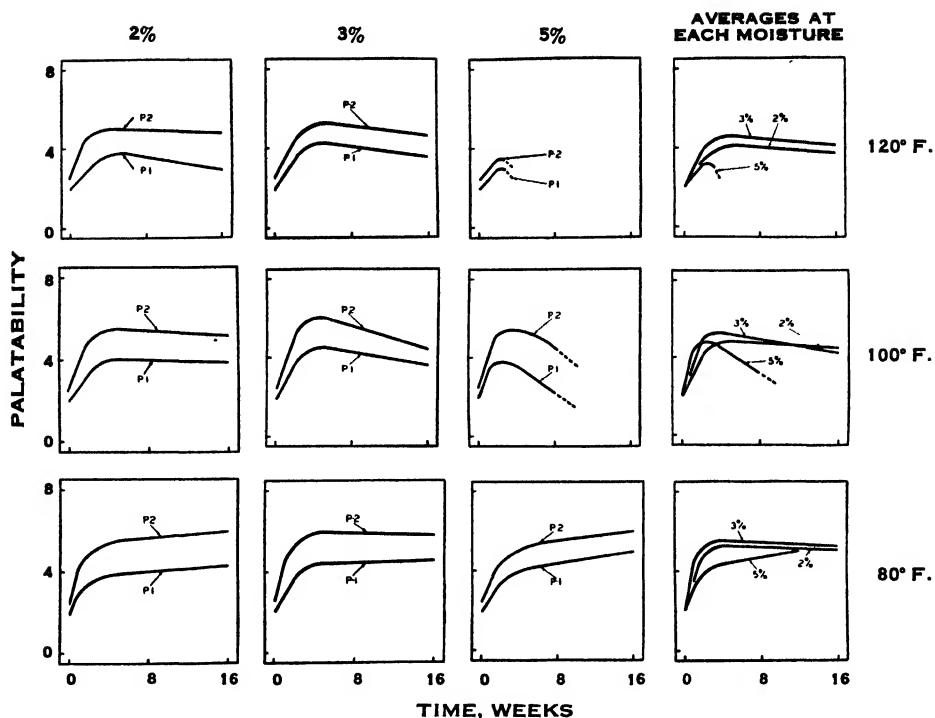
FIG. 6. *The effect of moisture content and storage temperature on the palatability of skim-milk powders from Plants 1 and 2.*

Discussion

This work has shown that considerable difference existed between plants in the effect of temperature on the keeping quality of whole milk powders. Deterioration was measurable at temperatures of 60° F., the rate of deterioration increasing with temperature and approximating a straight line relation. For skim-milk powders the marked difference in initial palatability between plants was uniformly maintained throughout the storage period. For both plants at all temperatures the palatability of skim-milk powders increased during the early part of the storage period.

Both whole and skim-milk powders at a moisture content of 3% maintained a higher palatability under storage than did these powders at moisture contents of 2 or 5%. Increased moisture contents were found to enhance the difference existing in the stability of powders from different plants.

The increase in palatability with storage of skim-milk powders has not been satisfactorily explained. It may be the result of the recombination in the powders of materials formed during early processing that was responsible for the low initial palatability. Protein degradation products such as various amines and dipeptides may recombine to form polypeptides whose flavours are not objectionable. At 80° F. this recombination may be sufficient to off-set the normal deterioration due to storage, and hence at this temperature

the palatability continues to increase throughout the entire storage period. Another explanation is that the concentration of these undesirable products may have been reduced by volatilization during storage (8). Further study of this problem is being made at the present time.

References

1. DAHLE, C. D. and PALMER, L. S. J. Dairy Sci. 7 : 40-57. 1924.
2. GREENBANK, G. R. and HOLM, G. E. Ind. Eng. Chem. 16 : 598-601. 1924.
3. HOLLENDER, H. A. and TRACY, P. H. J. Dairy Sci. 25 : 249-274. 1942.
4. HOLM, G. E., WRIGHT, P. A., and GREENBANK, G. R. J. Dairy Sci. 10 : 33-40. 1927.
5. LEA, C. H., MORAN, T., and SMITH, J. A. B. J. Dairy Research, 13 : 162-265. 1943.
6. PEARCE, J. A. Can. J. Research, F, 22 : 87-95. 1944.
7. PEARCE, J. A. Can. J. Research, F, 23 : 177-184. 1945.
8. PEARCE, J. A. and BRYCE, W. A. Can. J. Research, F, 23 : 334-339. 1945
9. SUPPLEE, G. C. Proc. World's Dairy Congr. 3 : 1248. 1923.
10. WOODCOCK, A. H. Can. J. Research, F, 23 : 117-122. 1945.

THE KEEPING QUALITY OF DEHYDRATED MIXTURES OF EGG AND MILK

By Jessie A. Pearce, Joan Whittaker, H. Tessier, and W. A. Bryce

THE KEEPING QUALITY OF DEHYDRATED MIXTURES OF EGG AND MILK[1]

By Jesse A. Pearce[2], Joan Whittaker[3], H. Tessier[4], and W. A. Bryce[2]

Abstract

The storage life of a dehydrated mixture of egg and milk, when assessed by both palatability and fluorescence measurements, was shorter than the life of milk powder of similar protein, fat, and carbohydrate content. Increased quantities of egg in the mixture decreased the quality of the mixture, both initially and during 16 weeks' storage. These effects were noticeable at all temperatures studied between 40° and 140° F. but were most marked above 80° F. After 16 weeks at 80° F., material packed under carbon dioxide usually had better palatability than the air-packed products. The effect of added sugar was most noticeable at 120° and 140° F. Lactose had a slightly beneficial effect; sucrose was more effective.

Introduction

Preliminary data obtained in these laboratories indicated that dehydrated mixtures of milk and egg were less stable during storage than were either of these components separately. This becomes a matter of importance if these dehydrated mixtures are to be prepared for satisfactory reconstitution into ice cream, custard mixes, milk shakes, or other similar foods. Mixes such as these are commercially available in Canada and are also being used to prepare ice cream for the American armed services (12). Possible use by the Canadian armed forces led to a study of their keeping quality.

The addition of sugar to milk before dehydration was believed to improve the keeping quality of the product (4) and the addition of sucrose to eggs before drying retarded fluorescence development (11), which in turn is related to egg powder quality (9). Therefore, the preservative effect of sucrose and lactose on these materials was evaluated, since products of the type described are usually used in the preparation of sweetened dishes and the addition of a small amount of sugar to the liquid before drying would not affect its use.

Packing milk powder under an atmosphere of carbon dioxide was reported to extend the storage life of this product (5), although recent evidence indicated that any beneficial effect was not evident to a taste panel (14). Egg powder when packed under this gas deteriorated less rapidly than air-packed material (8). It was desirable in this study to obtain additional information about the effect of gas-packing by comparing carbon-dioxide–packed with air-packed mixtures.

Materials and Methods

Mixtures Used and Storage Conditions

The materials used were obtained from commercial Canadian sources. Some formulas were prepared by mixing, and dehydrating, using a laboratory spray drier (15); others, by mixing the ingredients in the dry state. Differences in moisture content of dried egg powder (13) and dried milk powder (3) affect the keeping quality of both of these products. Therefore, the moisture content of the mixtures was kept within a range usually satisfactory for both dehydrated egg and milk (2 to 4%).

TABLE I

CALCULATED FAT, PROTEIN, AND CARBOHYDRATE PERCENTAGES OF VARIOUS SPRAY-DRIED EGG AND MILK MIXTURES

(Products at 2.5% moisture)

Mixture	Fat	Protein	Carbo-hydrate	Ash
(A) 2.4 lb. of skim-milk powder in 20 lb. of fresh whole milk	15	31	47	4.8
(B) 1 doz. fresh eggs, 2.4 lb. of skim-milk powder, in 20 lb. of fresh whole milk	20	35	38	4.4
(C) 5 doz. fresh eggs, 2.4 lb. of skim-milk powder in 20 lb. of fresh whole milk	30	42	22	4.0
(D) 1 lb. of lactose, 5 lb. of fresh eggs, 1 lb. of skim-milk powder in 15 lb. of fresh whole milk	21	30	41	4.5
(E) Dry mix of whole milk powder, whole egg powder, dried fermented egg albumen, and lactose in proportions of 147: 78: 76: 125	17	33	45	3.5
(F) Dry mix of whole milk powders, skim-powder and whole egg powder in proportions of 147: 200: 78	17	35	39	6.4

The formulas investigated and the calculated analyses are given in Table I. Formula *A* gave a mixture without egg; *B* gave a mixture with a moderate amount of egg; and *C* showed the effect of adding a large amount of egg; while *D* gave a mixture with a high egg level, but of a composition similar to *B*. Formula *F* provided a dry-mixture approximating *B*, and *E* was a similar dry-mix with skim-milk protein replaced by egg protein. The effect of sugar on the keeping properties of Formulas *A*, *B*, and *C* was evaluated by drying these mixes after the addition of 3% of lactose or 3% of sucrose. While these were only a few of the possible combinations they were representative of the products likely to be used.

All mixtures were packed in hermetically sealed tin plate containers with air in the headspace and stored at 40°, 60°, 80°, 100°, 120°, and 140° F. (4°, 16°, 27°, 38°, 49°, and 60° C.). Some were packed in tin plate under carbon dioxide and stored at 80° F.

Analytical Methods

The quality of products such as these is dependent primarily on their acceptability when reconstituted as milk shakes, custards, ice cream, or other similar dishes. Therefore, the average palatability score, determined by a panel of 14 tasters, was used for assessing quality. The material was reconstituted as a milk shake mix in a manner similar to that described for milk powder (7), but using one part solids and three parts of water by weight. The products when submitted to the tasters were sweetened but unflavoured, and to prevent differences in sweetness affecting tasters' judgment, all samples were made up to a level of 7% sweetness in terms of sucrose, utilizing recently tabulated sweetness relations (2).

The scoring technique differed from that used in previous work (7, 9). A freshly prepared mix of Formula *F* was used as the standard for tasting purposes and given an arbitrary score of 10. A score of 20 indicated a product twice as acceptable as this formula, a score of 5 indicated the product half as acceptable, and a score of 0 indicated an unacceptable mix. A fresh standard sample was used as a reference whenever palatability was determined.

An attempt was made to follow solubility changes in the mixtures by a standard procedure (1). However, during storage, the decrease in solubility of all products was so slight that the test was discontinued.

The fluorescence of a saline extract of defatted egg powder has been related to the palability of this product (9), and has also shown some relation to milk powder quality (6). Later work showed only a slight correlation between this test and the palatability of milk powder (7). Nevertheless, a modification of this test (10) was applied to some of the present samples. Correlation of fluorescence values of the stored mixes with the corresponding palatability scores was lower than that obtained for egg powder alone (9). This test was discontinued since the correlation was only .4 for the most desirable sample, Formula *A*. However, the fluorescence data were of some interest and are recorded here.

The data were subjected to analyses of variance and the significant effects were selected for discussion.

Results

Palatability Changes During Storage

The palatability changes in the basic mixtures during 16 weeks' storage at temperatures from 40° to 140° F. are shown in Fig. 1. Throughout the experiment the product prepared entirely from milk solids was considered superior, both initially and after storage. At 40° and 60° F., the mixture prepared entirely from milk and the dry mix of egg powder and whole and skim-milk powder deteriorated about equally, but less rapidly than other mixtures containing egg. At the higher temperatures (80 to 140° F.) all mixtures containing egg spoiled more rapidly than the mixture prepared only from milk. At temperatures of 100° to 140° F. the material containing the greatest quantity of egg became inedible more rapidly than those containing

little or no egg. The addition of egg to milk before dehydration reduced the initial quality of the dried product and its subsequent storage life. However, the dehydrated mixture appeared to have a better storage life than has been observed for egg powder (8).
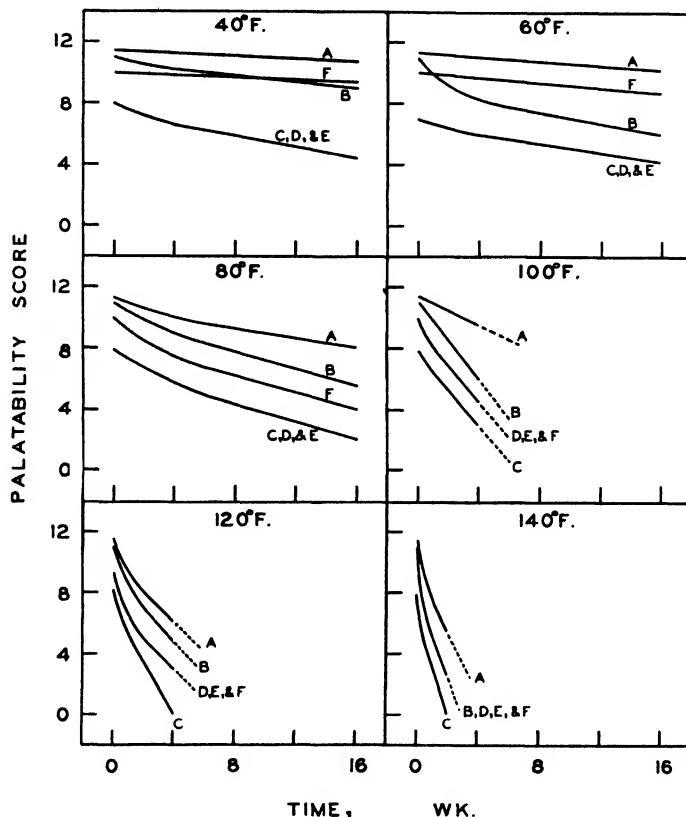
FIG. 1. *Effect of storage temperature on the palatability of stored dehydrated mixtures of egg and milk.*

The average values for samples with and without added sugars are shown in Fig. 2. Generally, there was a slight protective action as a result of the addition of sugar. The behaviour.was not regular at all temperatures nor for all mixes (notably Mixture *B* at the higher temperatures), nevertheless, added sucrose was more effective than added lactose.

Formula *D* was similar to Formula *C* since it gave a dried product with a large quantity of egg solids, and was similar to Formula *B* in that it yielded a product with almost the same fat, protein, and carbohydrate ratio. This mixture had slightly better keeping quality at the higher temperatures than Formula *C* (Fig. 1). It was believed that this improvement was the result of adding lactose rather than the result of balancing the component ratio.
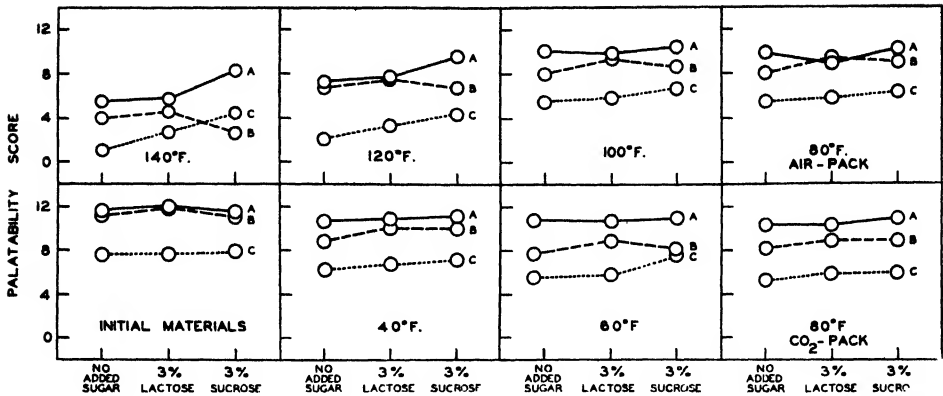
FIG. 2. *Average effect of added lactose and sucrose on the palatability of stored dehydrated mixtures of egg and milk.*

The comparative effects of packing some of the mixtures under atmospheres of air and carbon dioxide are shown in Fig. 3. It was observed that carbon dioxide provided some protection to the products *A*, *B*, and *D*, i.e., protection was afforded to mixtures of about the same composition even if eggs were
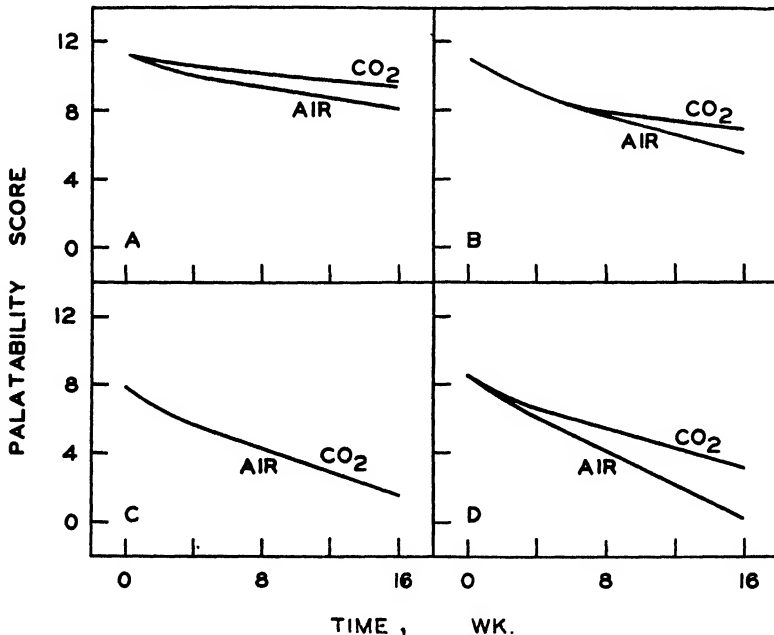
FIG. 3. *Effect on palatability of packing dehydrated mixtures of egg and milk under an atmosphere of carbon dioxide; storage at 80° F.*

present, but the life of material with high fat and protein from egg sources was not extended by carbon dioxide. The beneficial action of carbon dioxide was not pronounced; after 16 weeks' storage at 80° F. the palatability of

three of the gas-packed materials was about two units higher than that of the air-packed material.

Fluorescence Changes During Storage

As previously stated, fluorescence changes were measured during the initial portion of the experiment. In general, fluorescence increased with storage time and with temperature (Fig. 4). The initial fluorescence value was lower
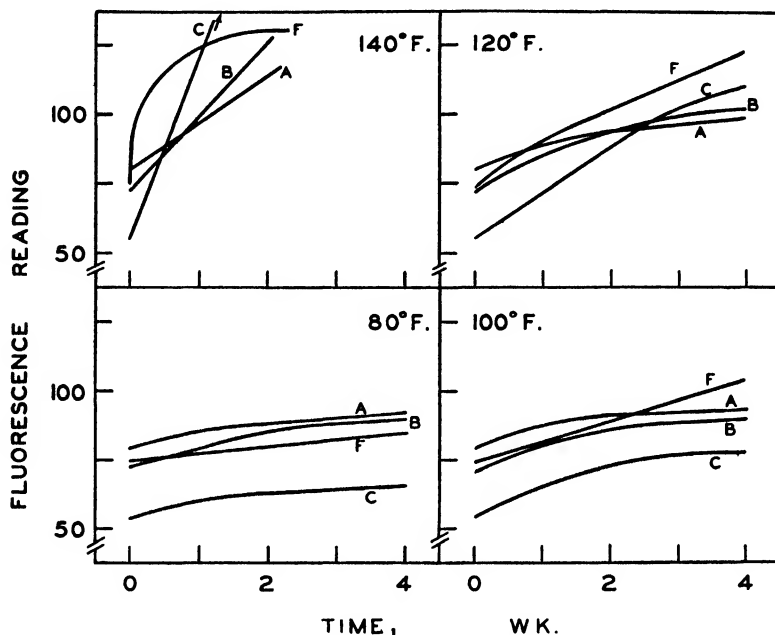
Fɪɢ. 4. *Effect of storage temperature on the fluorescence reading of saline extract of stored dehydrated mixtures of egg and milk.*

for mixes of higher egg content but the fluorescence value of these samples increased rapidly. Although parallel behaviour was usually noted for fluorescence increases and palatability decreases, the correlation between the two measures was less than that for egg powder alone (9).

One point of interest arises from comparison of Formulas *B* and *F*. At 100° to 140° F. the fluorescence of the dry-mix increased much more rapidly than that of the product prepared from the wet-mix, although the palatability measurements indicated about equal deterioration in these two materials.

Sucrose addition caused slight reduction in the average fluorescence value of the samples stored at temperatures of 80°, 100°, 120°, and 140° F. (Fig. 5). The greatest effect was apparent at 140° F.; sucrose caused the most pronounced reduction in fluorescence in the sample containing the greatest quantity of egg. This corresponds fairly well with similar observation from palatability scores (Fig. 2). The fluorescence measurement also indicated that sucrose was more effective than lactose.
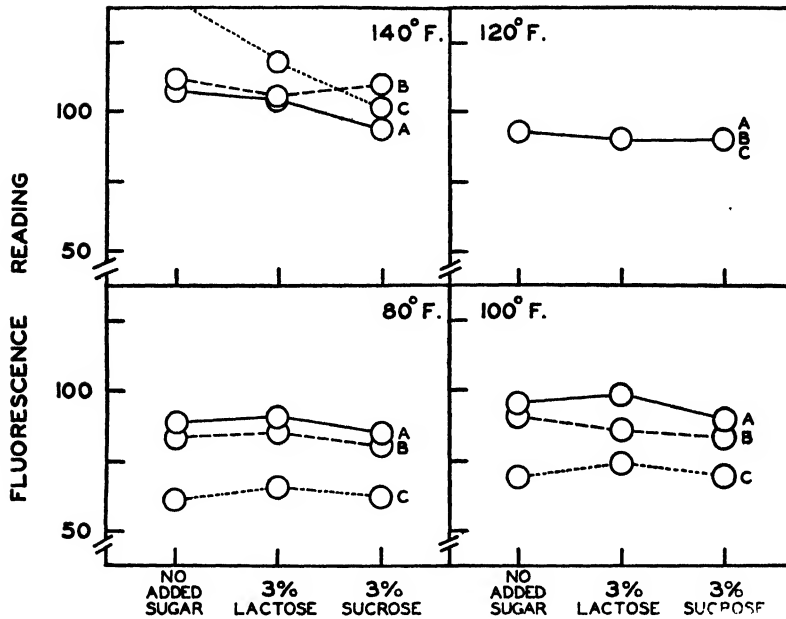
FIG. 5. *Average effect of added lactose and sucrose on the fluorescence reading of saline extracts of stored dehydrated mixtures of egg and milk.*

References

1. AMERICAN DRY MILK INSTITUTE, INC., CHICAGO, ILL.   The grading of dry milk powder. 1942.
2. CAMERON, A. T.   Can. J. Research, E, 22 : 45-63.   1944.
3. DAHLE, C. D. and PALMER, L. S.   J. Dairy Sci. 7 : 40-57.   1924.
4. DAVIES, W. L.   The chemistry of milk.   Chapman & Hall, Ltd., London.   1936.
5. LEA, C. H., MORAN, T., and SMITH, J. A. B.   J. Dairy Research, 13 : 162-215.   1943.
6. PEARCE, J. A.   Can. J. Research, F, 22 : 87-95.   1944.
7. PEARCE, J. A.   Can. J. Research, F, 23 : 177-184.   1945.
8. PEARCE, J. A., REID, M., and COOK, W. H.   Can. J. Research, F, 24 : 39-46.   1946.
9. PEARCE, J. A. and THISTLE, M. W.   Can. J. Research, D, 20 : 276-282.   1942.
10. PEARCE, J. A., THISTLE, M. W., and REID, M.   Can. J. Research, D, 21 : 341-347.   1943.
11. PEARCE, J. A., WOODCOCK, A. H., and GIBBONS, N. E.   Can. J. Research, F, 22 : 34-38. 1944.
12. U.S. ARMY QUARTERMASTER CORPS.   Tentative Specification C.Q.D. No. 141A.   5 Nov. 1943.
13. WHITE, W. H. and THISTLE, M. W.   Can. J. Research, D, 21 : 211-222.   1943.
14. WOODCOCK, A. H.   Can. J. Research, F, 23 : 117-122.   1945.
15. WOODCOCK, A. H. and TESSIER, H.   Can. J. Research, A, 21 : 75-78.   1943.

PACKAGING

III. EFFECT OF MOULD GROWTH AND AGEING ON THE WATER-VAPOUR TRANSMISSION OF PACKAGING MATERIALS[1]

By C. G. Lavers[2] and W. I. Illman[3]

Abstract

Packaging materials were dusted with mould spores and stored in a cabinet at 95° F. and 95 to 100% relative humidity for periods of one to eight weeks.

M.S.T. and M.S.A.T. "Cellophane" were attacked only slightly by mould, but deterioration of the heat-sealing, moisture-proof lacquer occurred during storage under conditions suitable for optimum mould growth. Moulds grew abundantly on M.S.Y.T. Cellophane. Wax-coated materials supported abundant mould growth, and their water-vapour transmission values increased when wax peeled from the surface of the sheet. The transmission rate of laminated materials having metal foil as one layer was not greatly affected by mould growth or delamination of the other layers. Abundant mould growth developed on most samples of kraft, and on glassine. Very little mould developed on cellulose acetate, Pliofilm, or vinyl-film.

Introduction

The effect of mould growth is undoubtedly an important factor in the deterioration of packaging materials if the atmosphere surrounding the package is of high humidity, or if the package contains material of high moisture content, i.e., material likely to be in equilibrium with high humidities. Concurrently with exposure to conditions favourable to the growth of moulds, changes may occur in packaging materials such as weakening of the fibre structure, delamination, or separation of a coating from the base material. Hence, the present investigation was undertaken to assess the effects of mould growth and ageing on the water-vapour transmission of a variety of packaging materials.

Materials and Methods

The materials used were various combinations of kraft paper, metal foil, glassine, "Cellophane," cellulose acetate, vinyl-film, and Pliofilm, both waxed and unwaxed. In addition, different grades and plies of Cellophane were investigated. When samples of duplex or triplex Cellophane were tested, the layers were heat sealed around the edge of the samples since these materials would be sealed in this manner when in use. Details of materials are noted in Table I.

The initial water-vapour transmission of the materials was determined. Samples were then dusted with mould spores of a variety of species and placed in a mould infested cabinet, operating at 95° F. and at 95 to 100% relative

humidity. Species included in the inoculum were; *Syncephalastrum racemosum, Paecilomyces variota, Penicillium* spp., *Aspergillus niger, Aspergillus flavus, Aspergillus terreus, Chaetomium globosum,* and *Stachybotrys atra.* Samples were removed after storage for one, two, four, and eight weeks, were inspected for mould growth and physical deterioration, and the water-vapour transmission determined. All measurements of water-vapour transmission' were made using a vapometer at a vapour pressure differential of approximately 26 mm. of mercury and a dry bulb temperature of 80° F. The vapometer was the same as that used in a previous study of packaging materials (2). It consisted of a flanged aluminium cup containing 10 ml. of water. Over the mouth of the cup the sample could be attached by another flange securely bolted to effect the seal. Although more accurate methods of measurement are desirable (1), this technique was satisfactory for the present purpose and provided a relative scale for comparing changes in water-vapour transmission occasioned by mould growth and ageing.

Results

The results are shown in Figs. 1 to 20, in which the figure numbers correspond with the material numbers given in Table I. All water-vapour transmissions plotted are averages of triplicate determinations. The severity of attack by moulds was classified into four groups: none (*N*), surface free of mould growth; slight (*S*), growth had started at particles of foreign matter on the surface of the material; medium (*M*), growth was more general and moulds were actually feeding on the packaging material; abundant (*A*), growth was heavy and general.

M.S.T. and M.S.A.T. Cellophane were only slightly attacked by moulds except in spots where the lacquer had been damaged by heat sealing (Figs. 1, 2, 3, and 5). It is believed that the fairly large increase in the water-vapour transmission of these materials was due to loosening and cracking of the heat-sealing lacquer brought about by the severe conditions of storage, rather than to mould growth. In contrast to the above grades, M.S.Y.T. Cellophane (Fig. 4) developed an abundant growth of mould over its entire surface. Possibly a difference in the processing of this type of film caused this greater proliferation of organisms. There was a much smaller increase in the transmission of duplex and triplex Cellophanes (Figs. 2, 3, 4, and 5) than in that of the single sheet (Fig. 1). This probably occurred because some protection was afforded the lacquer on the inner faces of the Cellophane.

Abundant mould growth occurred on all materials that were wax-impregnated or wax-coated (Figs. 6 to 10). Wax-impregnated kraft (Fig. 6) showed a large increase in water-vapour transmission concurrent with abundant mould development. Wax-coated kraft (Fig. 7) suffered only a slight increase in water-vapour transmission, and only a slight separation of the wax from the kraft. Wax-coated Cellophanes (Figs. 8 and 9), however, showed large increases in their water-vapour transmission, and the wax tended to peel from the Cellophane. The increased transmission values of wax-coated

TABLE I

PACKAGING MATERIALS STUDIED

Material No.	Description of material
	Cellophanes
1	300 M.S.A.T., single
2	300 M.S.T., duplex
3	300 M.S.A.T., duplex
4	300 M.S.Y.T., duplex
5	300 M.S.A.T., triplex
	Waxed materials
6	40 Lb. kraft, wax impregnated
7	40 Lb. wet strength kraft, wax coated* 40 lb./ream
8	300 M.S.T. Cellophane, wax coated* 40 lb./ream
9	300 M.S.T. Cellophane laminated to 300 M.S.T. Cellophane, wax coated* 40 lb./ream
10	300 M.S.A.T. Cellophane laminated to scrim. Cellophane lightly waxed, scrim heavily waxed*
	Laminated materials
11	300 M.S.T. Cellophane laminated to 300 M.S.T. Cellophane
12	300 M.S.T. Cellophane laminated to metal foil
13	300 M.S.A.T. Cellophane laminated to 25 lb. kraft.
14	40 Lb. kraft laminated to alloyed lead foil laminated to 300 M.S.T. Cellophane
15	Scrim laminated to kraft laminated to alloyed lead foil with butvar coating
16	25 Lb. kraft laminated to cellulose acetate
17	25 Lb. kraft laminated to 25 lb. bleached glassine
18	25 Lb. bleached glassine laminated to 25 lb. bleached glassine
19	Vinyl-film laminated to both sides of 0.001 in. aluminium foil
	Pliofilm
20	Pliofilm, single

* *Flexible wax compound.*

materials were probably the result of this loosening of the wax coating. The data show the extent of the reduction in water-vapour transmission resulting from wax application, and its subsequent increase with separation of the wax from the base stock. Scrim laminated to Cellophane and heavily waxed (Fig. 10) supported abundant mould growth and showed a gradual but consistent increase in water-vapour transmission.

The water-vapour transmission of laminated materials having metal foil as one layer was not greatly affected even though moulds grew abundantly on the other layers and considerable delamination occurred. Examples of this were: Cellophane laminated to metal foil (Fig. 12), where considerable delamination occurred, and kraft laminated to foil laminated to Cellophane (Fig. 14), where considerable delamination of the Cellophane and slight delamination of the kraft occurred. Also, the foil blistered slightly on samples of foil laminated to kraft backed with scrim (Fig. 15). Very little mould grew on vinyl-film laminated to aluminium foil (Fig. 19) and very slight delamination occurred. This very low increase in water-vapour transmission indicated the general desirability of using a foil type barrier when
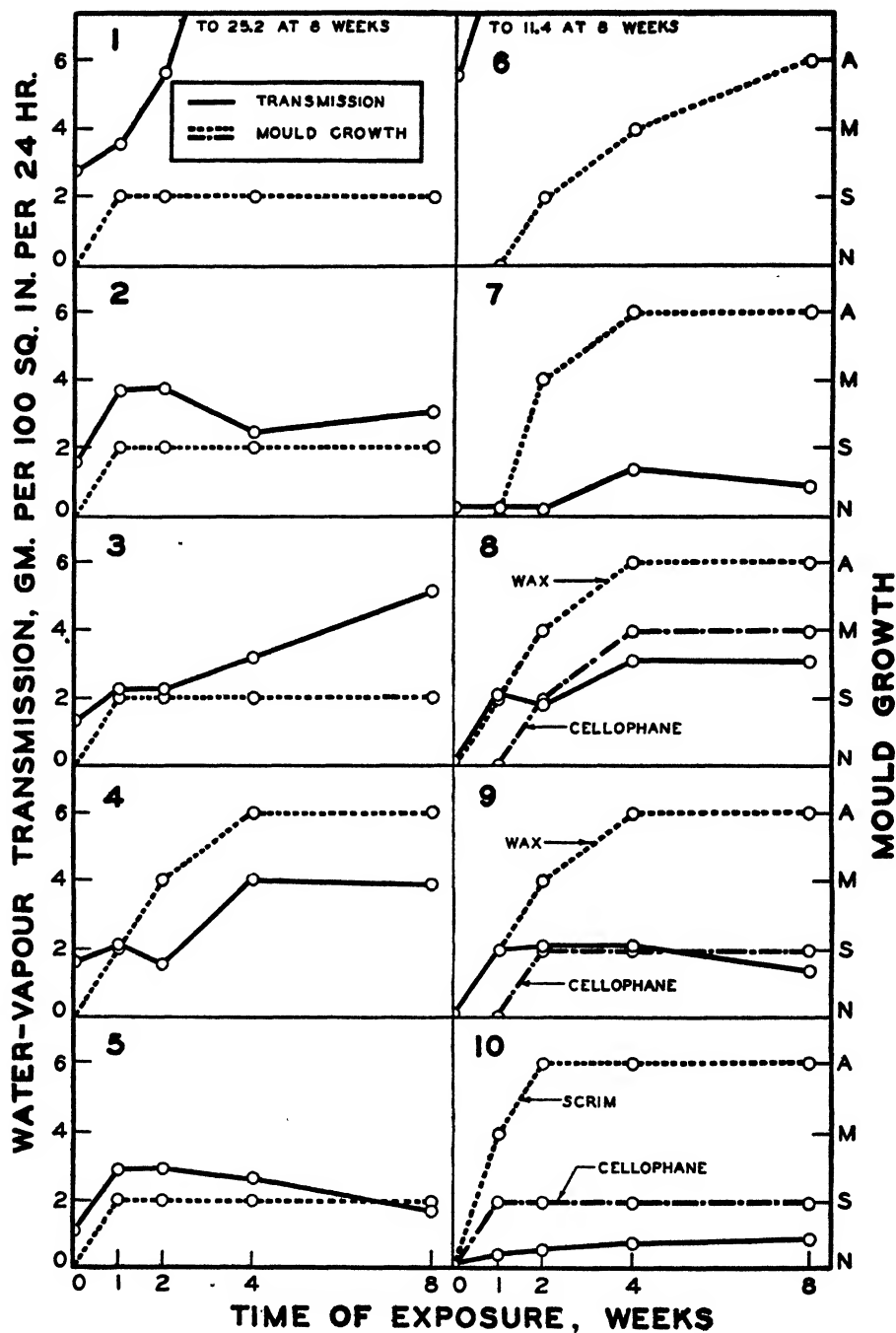
FIGS. 1-10. *Effect of mould growth and ageing on the water-vapour transmission of Cellophane and of waxed packaging materials.* *(See Table I for key).*
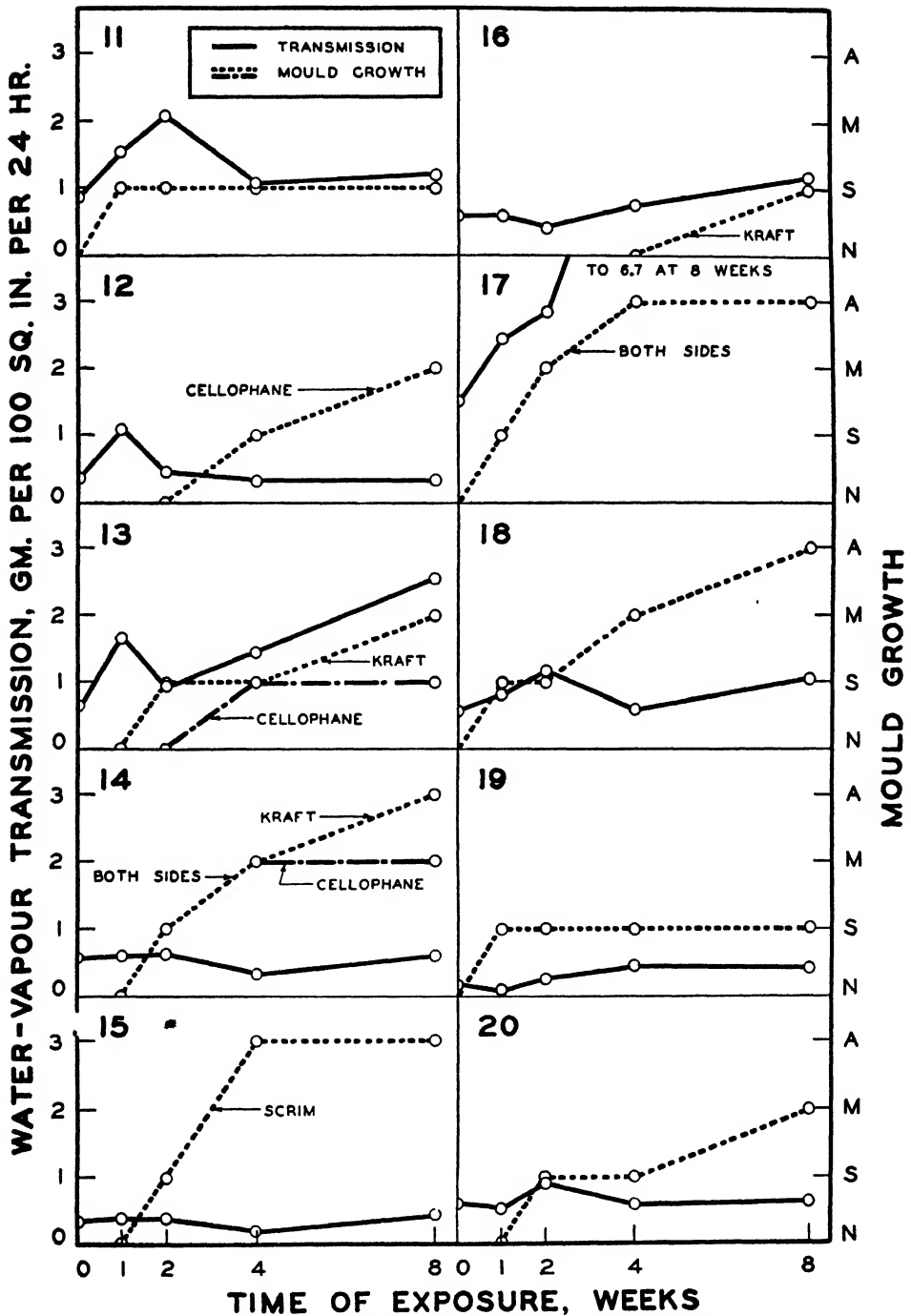
FIGS. 11-20. *Effect of mould growth and ageing on the water-vapour transmission of laminated packaging materials and of Pliofilm. (See Table I for key).*

severe conditions of temperature and humidity are likely to be encountered. Equal protection might not be provided, however, in a completed package, since the barrier would be of little value if the material forming the heat-sealing surface loosened from the foil.

Most of the materials having kraft paper as an outer layer developed abundant mould growth on the kraft side. In addition to supporting a heavy growth of moulds on the kraft side, samples of Cellophane laminated to kraft (Fig. 13) delaminated considerably and the water-vapour transmission showed a significant increase. No mould grew on the cellulose acetate sheet laminated to kraft (Fig. 16), and only a slight amount on the kraft. The acetate sheet appears to have inhibited growth on the kraft. Moulds grew abundantly on kraft laminated to glassine (Fig. 17) and caused marked deterioration, penetrating the sheet completely at one point. Laminated glassine itself (Fig. 18) supported abundant mould growth, but the increase in water-vapour transmission was much less than that of glassine laminated to kraft.

Pliofilm (Fig. 20) showed only slight mould growth, and little increase in water-vapour transmission.

References

1. CHARCH, W. H. and SCROGGIE, A. G.   Paper Trade J. 101 : 201-209.   1935.
2. WOODCOCK, A. H., CHAPMAN, M. G., and PEARCE, J. A.   Can. J. Research, F, 23 : 109-116.   1945.

DRIED MILK POWDER

V. THE PHOTOLYSIS OF RIBOFLAVIN IN MILK POWDERS[1]

By W. A. Bryce[2]

Abstract

Exposure of milk powders to sunlight resulted in a much greater destruction of riboflavin than did exposure to ultra-violet light in the range 3200 to 4200 Å. The rate of photolysis was greater for skim-milk powders than for whole milk powders. Increased intensities of visible light accelerated riboflavin destruction. In the spectral region of 4200 to 5600 Å the wave band causing the greatest destruction in liquid skim-milk had a principal wave-length of 4450 Å, which corresponded to a maximum in the absorption spectrum of riboflavin. The rate of photolysis of riboflavin was a function of both wave-length and intensity of the impinging energy.

Introduction

Riboflavin, or vitamin B_2, is a valuable component of foodstuffs. It is effective in promoting growth in rats, and in preventing lesions of cheilosis and nutritional anemia in humans (11). It is also an important factor in cell respiration and metabolism (11). It is stable to heat, but is readily decomposed when exposed to light (4). Since milk is an important source of riboflavin, the photochemical decomposition, or photolysis, of this vitamin in milk has been studied. Liquid whole milk in commercial milk bottles exposed to summer sunlight for two hours lost two-thirds of its riboflavin (6, 9, 15). Sunlight apparently causes much greater destruction of riboflavin than ultra-violet light, as it has been reported that commercial irradiation of milk sufficient to produce a vitamin D content of 400 U.S.P. units per quart had no effect on the riboflavin content (3). Another investigator reported a slight decrease in riboflavin content of milk as a result of commercial irradiation (16).

Increases in either alkalinity or temperature accelerate the photolysis of riboflavin in liquid whole milk. Temperature and pH are believed to affect the photodecomposition rather than other chemical decompositions, for when riboflavin solutions are heated in the dark at 100° C. under mild acid conditions for four hours, little or no destruction of the vitamin occurs (14).

Since milk powder may be exposed to light during post-drying handling and storage and while it is being prepared for consumption, it was felt that some information on the photolysis of riboflavin in milk powders would be of value. This paper describes an investigation designed to study the effect of sunlight and ultra-violet light on the riboflavin content of milk powders of various fat contents obtained from different plants. It also includes some incidental measurements on riboflavin destruction in liquid skim-milk and on light absorption by riboflavin.

Materials and Methods

The powders used in the study were the commercial spray-dried products of two Canadian companies.   Powders of 1, 26, 28, and 30% butter fat were obtained from one plant, and of 1, 26, and 28% butter fat from another, all powders having a moisture content of 3.0%.

All riboflavin determinations were made using the photofluorometric method developed by Hodson and Norris (5).   After the initial riboflavin contents were determined, samples of the powders were placed in sealed Pyrex glass containers, and were analysed for riboflavin content after exposure to sunlight for 60, 110, and 215 hr. (the latter period corresponding to 48 days' storage) at an average temperature of 6.1° C. (43° F.).   The average light energy falling on the powders was assumed to be 1.8 cal. per sq. cm. per min.   During the study the containers were shaken by hand every three days in an attempt to ensure that all of the powder would be exposed.   Control samples of the powders were stored in light-proof containers at the same temperature.

Additional samples of the same powders were exposed in Pyrex glass containers at a distance of 6 in. from a 250 watt Purple-X bulb that emitted ultra-violet light in the range 3200 to 4200 Å.   The energy falling on the samples was calculated to be approximately 0.0034 cal. per sq. cm. per min. Riboflavin determinations were made after periods of 75, 150, and 300 hr. (42 days' storage).   The temperature during this phase of the study was 38° C. (100° F.).   The powders were shaken after each 10 hr. of exposure. Control samples in light-proof containers were stored under the same conditions.

The effect of variations in 'light' intensity on the rate of photolysis of riboflavin in milk powders was also studied, as calculations on the energies falling on the samples from the sun and from the ultra-violet bulb showed great differences between the two sources.   Samples of whole and skim-milk powders were sealed in flat glass containers, and were exposed for 75 hr. at distances of 1 ft. and 4 ft. from a 100 watt tungsten lamp.   The energy intensities at the two levels were 0.0036 and 0.0004 cal. per sq. cm. per min., respectively.   The destruction of the riboflavin at each intensity of light was determined.

It was recognized that the riboflavin destruction caused by different parts of the near ultra-violet and visible spectrum might not be equal, and therefore an attempt was made to determine which bands were the most destructive. Samples of fresh skim-milk in glass cuvettes were exposed to successive wave bands from 4200 to 5600 Å, all bands being 200 Å wide and of equal intensity. The bands were obtained by the use of Corning glass filters.   The intensities, measured by means of a Weston exposure meter, were adjusted to a constant value by varying the potential applied to the tungsten lamp used as the light source.   After exposure for four hours the riboflavin content of the milk samples was determined.

The absorption spectrum of riboflavin in aqueous solution was also determined for the above light range, as it was believed that a direct relation

should exist between the wave-lengths causing maximum destruction and those at which the greatest absorption occurred. A Beckman Quartz Spectrophotometer was used to determine the absorption spectrum.

Control samples of powders in light-proof containers were maintained under comparable storage conditions whenever powders were exposed to visible or ultra-violet light. No decrease in riboflavin content occurred in any of these control samples during storage.

Results

The data for whole and for skim-milk powders were assessed by analysis of variance. No difference was observed between samples of whole or between samples of skim powders exposed to sunlight. The time of exposure had an effect on the whole milk powders but any change in skim powders was not assessed as significant. The results for samples exposed to sunlight are shown in Fig. 1. Marked destruction of the riboflavin occurred during the first 60
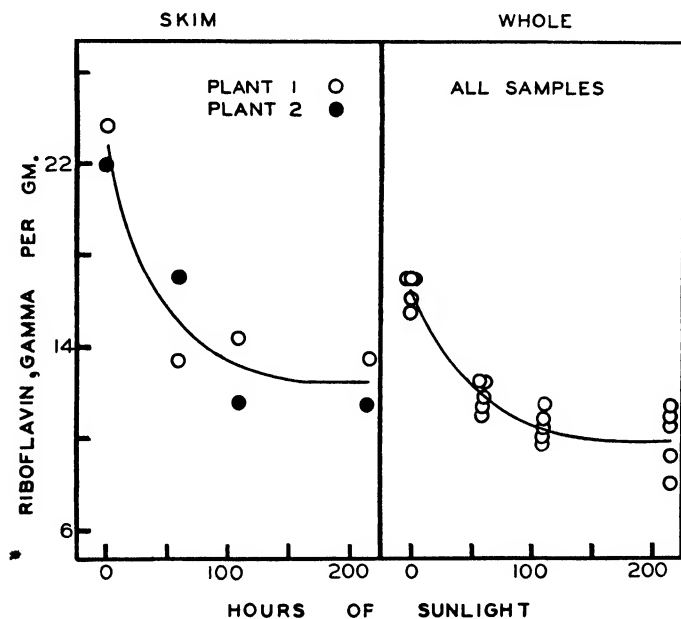
FIG. 1. *The effect of sunlight on the destruction of riboflavin in milk powders from Plants 1 and 2.*

hr., after which the rate of photolysis was greatly reduced. Skim-milk powders had higher riboflavin contents than whole milk powders and suffered greater destruction than the whole powders. Vitamin losses occurred at approximately equivalent rates in the skim-milk powders from the two plants, the initial differences being largely maintained throughout.

Similar mathematical assessment of the data for powders exposed to ultraviolet light showed that the only significant difference was that between skim-milk powders from the two producers. Nevertheless, there was some evidence

of riboflavin destruction (Fig. 2). The rate of destruction was about the same for both types of powders, since the curves for the skim and whole milk powders were practically parallel. The most pronounced difference in these comparisons was that resulting from exposure to the two different light sources. Sunlight caused a greater decrease in riboflavin content than ultraviolet light.
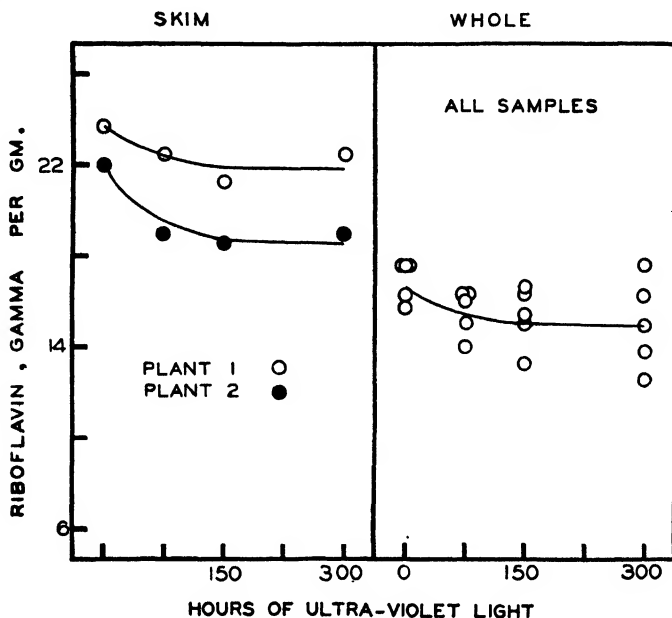
FIG. 2. *The effect of ultra-violet light (3200 to 4200 Å) on the destruction of riboflavin in milk powders from Plants 1 and 2.*

Variations in the intensity of the energy falling on the samples had a marked effect on the rate of photolysis of the riboflavin in skim-milk powders as is evident from the data in Table I and from a comparison of Figs. 1 and 2. In whole milk powders, the differences in energy did not greatly affect the rate of destruction of the riboflavin.

TABLE I

EFFECT OF VARIATION IN INTENSITY OF LIGHT ON DESTRUCTION OF RIBOFLAVIN; 75 HR. EXPOSURE TO A 100 WATT TUNGSTEN LAMP

Distance from lamp, ft.	Energy level, cal./sq. cm./min.	Riboflavin content (gamma/gm.)			
		Skim-milk powder		Whole milk powder	
		Initial	Final	Initial	Final
1	0.0036	22.00	12.50	17.50	10.00
4	0.0004	22.00	17.00	17.50	11.75

The data for the comparison of the destructive effect of wave bands between 4200 and 5600 Å are shown in Fig. 3. While all wave bands studied caused some destruction of the riboflavin, the greatest decrease occurred at the band having a principal wave-length of 4450 Å. From Fig. 4 it can be seen that this wave-length corresponds approximately to a maximum in the absorption spectrum of riboflavin.
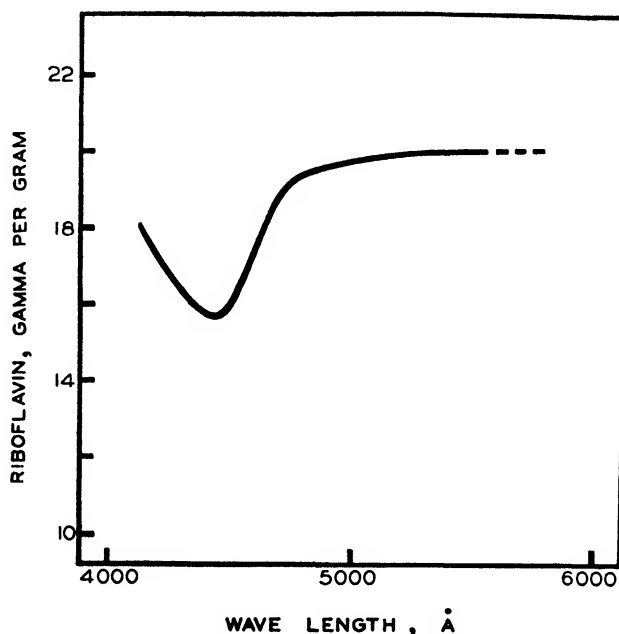
FIG. 3. *The effect of various wave-lengths of light on the destruction of riboflavin in liquid skim-milk.* (*Riboflavin content calculated on basis of milk solids; initial value, 22 gamma per gm.*).

Discussion

The destruction of riboflavin in the milk powders was much more rapid and complete owing to exposure to sunlight than it was as a result of exposure to ultra-violet light. This was believed to be due to the fact that the energy in the sunlight was much greater than that from the ultra-violet source, and also because the most destructive wave-lengths were not present in the ultra-violet light used in this experiment. The data in Table I showed that the greatest destruction occurred in the powders at the greatest level of light intensity. The effect was much more noticeable for skim-milk powders than it was for whole milk powders.

The wave-length of the incident light was an important factor in the photolysis of riboflavin. The marked destruction of the vitamin due to sunlight action was probably partially attributable to the presence in sunlight of the destructive wave band around 4450 Å. Further evidence of the significance of wave-length of the impinging light on the rate of riboflavin photolysis was

provided by the observation that when skim-milk powder was exposed to visible light and to ultra-violet light of equal intensity (0.0034 cal. per sq. cm. per min.) for the same length of time (75 hr.), 43% of the riboflavin was destroyed by the visible light, and only 10% was destroyed by the ultra-violet light. Only in the visible light was the destructive wave band around 4450 Å present. The fact that commercial irradiation of milk causes only a slight decrease in riboflavin content is possibly due to the absence of these destructive wave-lengths from the ultra-violet light used.
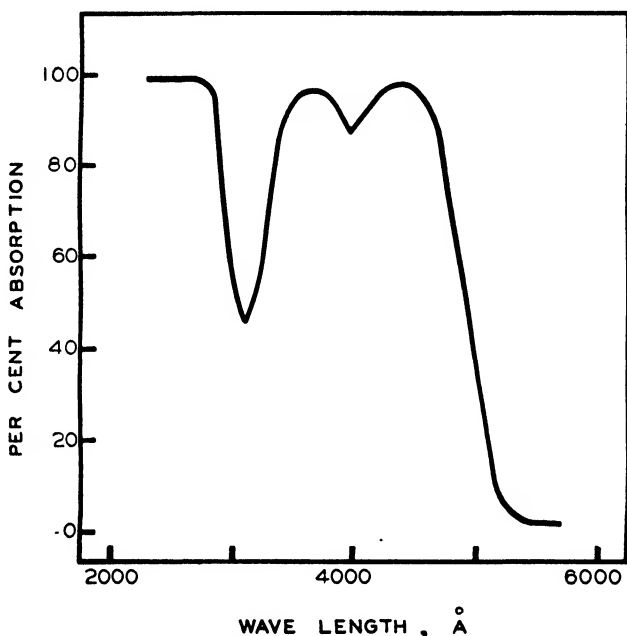
FIG. 4. *The absorption spectrum of riboflavin in the range 2500 to 5500 Å.*

The apparent decrease in the rate of destruction of riboflavin in the milk powders after prolonged exposure to sunlight has also been observed during the photolysis of alkaline solutions of pure riboflavin*. In the latter study, the results obtained from photofluorometric assays were not confirmed by microbiological determinations. Materials with chemical and fluorescent properties similar to those of riboflavin may have been measured as riboflavin in the photofluorometric analysis. Irradiation products of riboflavin have been isolated and identified (7, 8) and the fluorescent spectrum of one of these substances, lumiflavin, is similar to that of the vitamin (1, 2, 7, 13).

The decrease in rate of photolysis may also be attributed to the formation of substances that protected the vitamin from further photolysis. This problem is receiving attention in these laboratories at the present time.

* *Gorham, P. Unpublished results.*

Acknowledgments

References

1. BIERRY, H. and GOUZON, B.   Documentation sci. 5 : 309-318.   1936.
2. BIERRY, H. and GOUZON, B.   Documentation sci. 6 : 17-27.   1937.
3. FUHR, I., DORNBUSH, A. C., and PETERSON, W. H.   J. Dairy Sci. 26 : 643-646.   1943.
4. GYÖRGY, P., KUHN, R., and WAGNER-JAUREGG, T.   Naturwissenschaften, 21 : 560-561   1933.
5. HODSON, A. Z. and NORRIS, L. C.   J. Biol. Chem. 131 : 621-630.   1939.
6. HOLMES, A. D. and JONES, C. P.   J. Nutrition, 29 : 201-209.   1945.
7. KARRER, P., SALOMON, H., SCHÖPP, K., SCHLITTLER, E., and FRITZSCHE, H. Helv. Chim. Acta, 17 : 1010-1013.   1934.
8. KUHN, R. and MORUZZI, G.   Ber. 67B : 1220-1223.   1934.
9. PETERSON, W. J., HAIG, F. M., and SHAW, A. O.   J. Am. Chem. Soc. 66 : 662.   1944.
10. ROSCOE, M. H.   Biochem. J. 27 : 1540-1544.   1933.
11. ROSENBERG, H. R.   Chemistry and physiology of the vitamins.   Interscience Publishers Inc., New York.   1942.
12. THEORELL, H.   Biochem. Z. 279 : 186-200.   1935.
13. WARBURG, O. and CHRISTIAN, W.   Biochem. Z. 258 : 496-498.   1933.
14. WILLIAMS, R. R. and CHELDELIN, V. H.   Science, 96 : 22-23.   1942.
15. ZIEGLER, J. A.   J. Am. Chem. Soc. 66 : 1039-1040.   1944.
16. ZIEGLER, J. A. and KEEVIL, N. B.   J. Biol. Chem. 155 : 605-606.   1944.

LIQUID AND FROZEN EGG

I. APPLICATION OF A FLUORESCENCE MEASUREMENT TO LIQUID AND FROZEN EGG

BY MARGARET REID

LIQUID AND FROZEN EGG

I. APPLICATION OF A FLUORESCENCE MEASUREMENT TO LIQUID AND FROZEN EGG[1]

By Margaret Reid[2]

Abstract

The direct measurement of fluorescence on clarified sera collected as exudate from defrosting egg distinguished between samples of frozen egg of varying quality. The method may be useful in egg drying establishments where large quantities of frozen egg are available for test purposes.

A similar serum was extracted from liquid and frozen egg by the following procedure. Approximately 50 ml. of fresh or thawed egg liquid was added to approximately 100 ml. of chloroform, mixed by gentle swirling, allowed to stand two minutes, shaken vigorously for 30 sec., and immediately centrifuged for 15 min. at 2000 r.p.m. The top layer, consisting of 15 to 20 ml. of red serum, was poured off and clarified by centrifuging for one minute, and the fluorescence of the clear serum determined. The latter measure consistently distinguished between samples of liquid and frozen egg of varying quality. The serum produced was less highly fluorescent than that obtained as exudate, and consequently the range between quality types was narrowed.

The sera extracted with chloroform from freshly broken eggs of good quality had a higher fluorescence than that extracted from the material after standing at temperatures of 30° to 90° F. for short periods. However, the fluorescence rose when deterioration became evident. Freezing of liquid egg resulted in a decrease in fluorescent materials.

Introduction

The seasonal production of eggs, coupled with large wartime commitments, has made it necessary to store unprecedented quantities in the form of frozen blocks. No suitable test has been available for estimating the quality of liquid or frozen egg. During a preliminary investigation the following methods were tried without success: reducing sugar values measured on a tungstic acid filtrate (5, pp. 416 and 438); acid-soluble phosphorus (5, p. 461); peroxidase value (3); the fluorescence (6) of tungstic acid and trichloracetic acid filtrates of egg melange (5, pp. 416 and 461); pH (8); fluorescence of the liquid egg, a modification of a technique applied to egg powder (4); loaf volume (7); foaming volume (7); and solids content (1). Palatability ratings on the frozen egg liquid, cooked as scrambled egg and scored by the methods used for reconstituted dried egg powder (6), were also of little value in distinguishing quality. Eggs frozen from material discarded as infertile after 7 to 10 days' incubation were frequently preferred to a Grade A product.

During the course of these investigations, it was observed that a pink watery serum separated from frozen egg while defrosting. A similar serum could be extracted from both the fresh and defrosted products by shaking with chloroform and centrifuging. Measurements of refractive index, pH,

reducing sugar, and fluorescence were made directly on this extracted serum. Of these, only fluorescence showed promise as a possible measure of quality. The present paper describes the results to date of applying the fluorescence test to sera from samples of liquid and frozen egg of varying quality.

Materials and Methods

Materials

The various qualities of liquid and frozen egg used are described in the tables.

Methods

In preliminary work, serum was obtained by collecting the exudate from thawing egg. This material was clarified by centrifuging for one minute at 2000 r.p.m., and a fluorescence measurement was made on the supernatant fluid by methods previously described (6). However, 4 to 6 lb. of frozen egg was required to produce sufficient serum, and, moreover, no serum separated from egg frozen and stored for less than a month.

Separation of a similar serum from all types of liquid and frozen egg was brought about by adding chloroform to the egg, shaking, and centrifuging. In the adapted procedure, approximately 50 ml. of fresh or thawed egg liquid, which had been well mixed and strained, was added to approximately 100 ml. of chloroform, mixed by gentle swirling to avoid emulsification, allowed to stand for two minutes, shaken vigorously for 30 sec., and immediately centrifuged for 15 min. at 2000 r.p.m. The top layer, consisting of 15 to 20 ml. of red serum, was poured off and clarified by centrifuging for one minute. The fluorescence of the clear serum was then determined.

Results

The results are given in Tables I to IV, in which the samples are listed in order of decreasing quality.

In Table I the fluorescence values of sera obtained by seepage are compared with those of sera obtained by the chloroform extraction of materials representing a wide range of quality. It will be noted that both methods showed an increase in fluorescence values as quality decreased. Fluorescence values for sera obtained by seepage were consistently higher than the values for chloroform-extracted sera. Chloroform extraction appeared to remove approximately one-third of the fluorescing materials. The higher fluorescence values for sera collected by seepage resulted in better differentiation between quality types; nevertheless the chloroform extraction procedure was used in the subsequent work reported here because of its economy of time and materials.

Table II shows the effect of storing egg liquid at temperatures ranging from 30° to 90° F. Although fluorescence of the sera increased with increasing storage temperature, storage at the lower temperatures apparently resulted in an initial decrease in fluorescing materials. This behaviour was supported

TABLE I

FLUORESCENCE OF SERA OBTAINED FROM FROZEN EGG AFTER THREE MONTHS' STORAGE

(Means of six measurements)

Description of sample	Fluorescence	
	Sera extracted with chloroform	Sera obtained by seepage
Grade *A* (no off-odour)	6.9	9.4
Grade *C* (definite off-odour)	7.6	11.9
Grade *C* held in shell at room temperature for three weeks prior to freezing (definite off-odour)	8.3	17.5
Incubator rejects* (strong off-odour)	21.4	33.0
Grade *A* broken out and held at 80° F. for 36 hr. prior to freezing (extreme off-odour)	38.8	43.7

' *Infertile eggs rejected during incubation.*

TABLE II

EFFECT OF TEMPERATURES ABOVE FREEZING ON FLUORESCENCE OF SERA EXTRACTED FROM LIQUID EGG STORED FOR 32 HR.

(Means of two measurements)

Description of sample	Fluorescence of serum
Initial, before storage	10.0
Temperature of storage, ° F.	
30*	6.0
40	7.0
50	7.5
60	8.8
70	10.0
80	13.3
90	16.5

* *Held for 12 hr. only.*

by a subsequent observation that freshly broken eggs of good quality had a higher fluorescence than the same material after standing at 80° F. for 16 hr. in sterile containers (see Table IV).

Similar behaviour is shown in Table III. Sera extracted from fresh Grade *A* eggs before freezing had a higher fluorescence value than sera similarly extracted from eggs candled as Grade *A* after five months' commercial storage in the shell. It will be noted on examination of the standard deviations that

the variations between samples within each quality type were wide. This was not evident however where large batches of liquid egg were involved (Tables I and IV). It will also be observed in Table III that freezing consistently resulted in decreased fluorescence values.

TABLE III

Mean fluorescence and standard deviations for sera extracted from egg before and after freezing

(Means of 12 measurements)

Description of sample	Fluorescence			
	Before freezing		After freezing	
	Mean	Standard deviation	Mean	Standard deviation
Grade *A*, one day old	12.4	1.63	7.1	0.60
Grade *A*, stored five months at 30° F.	10.9	1.08	10.0	1.06
Grade *C*, stored two months at 30° F.	14.1	2.63	10.6	1.39
Incubator rejects, candled at four and seven days, held one week at 30° F.	17.7	2.53	15.7	1.22

Table IV shows the fluorescence values for sera obtained from Grade *A* liquid egg, from similar material after standing, and from Grade *C* and lower grade materials. The fluorescence of serum extracted from freshly broken liquid egg distinguished between quality types, Grade *C* melange being well differentiated from Grade *A*. No deterioration could be detected either organoleptically or by the fluorescence measurement in liquid egg stored 16 hr. at 80° F. in sterile glass (see also Table II).

These materials were then frozen and stored in the frozen state. Directly after freezing, the range in terms of fluorescence units was greatly narrowed—only two units separated Grade *A* and Grade *C* material. After storage at 10° F., fluorescence values for Grade *C* and cracked eggs were lowered still further. However, this drop did not appear in the materials stored at 0° and −10° F. (the temperatures used commercially) with the result that differentiation was equivalent to that for freshly frozen material. As measured by fluorescence, only minor changes in quality occurred in frozen egg stored for six months at temperatures ranging from −10° to 10° F.

Discussion

Fluorescence measurements on the serum exuded by thawing egg are considered to have distinct promise as a quality control measure in egg drying

TABLE IV

FLUORESCENCE VALUES OF SERA EXTRACTED FROM EGG MATERIALS OF VARYING QUALITY
BEFORE AND AFTER FREEZING, AND AFTER STORAGE IN THE FROZEN STATE

(Means of two measurements)

Treatment	Quality of materials used						
	Fresh Grade A* (no off-odour)	Grade A held 16 hr. at 80° F. in sterile glass prior to freezing (no off-odour)	Grade C (slight off-odour)	Cracked eggs (definite off-odour)	Musty eggs (strong off-odour)	Incubator rejects (very strong off-odour)	Mean values for treatments
Before freezing	10.0	6.3	14.0	15.0	32.0	58.2	22.6
After freezing	6.1	5.6	8.0	11.6	14.5	32.5	13.1
Three months storage at 10° F.	6.1	6.4	7.0	8.5	14 0	27.0	11.5
0° F.	6.5	7.0	8.6	8.9	14 0	26.0	11.8
−10° F.	6.7	7.2	9 4	11.8	16.4	31.0	13.8
Six months storage at 10° F.	6.3	6.5	7.0	9 7	15 9	33.0	13 1
0° F.	6.3	6.2	7.6	11.5	15.2	30.5	12.9
−10° F.	6.6	6.5	7.8	9 5	17.0	35.5	13.8
Mean values for quality types	6.8	6.5	8.7	10.8	17 4	34.2	—
Duplicate error	± 0.3			± 1.0			

* *Means of six measurements.*

plants. The fact that no serum separates from frozen egg stored for less than a month, and that 4 to 6 lb. is required for a measurement, make the measure unsuitable for most investigational purposes—but these restrictions do not apply to quality control in egg drying establishments, since the frozen egg is usually stored for more than a month before drying, and all the frozen material has to be thawed in any event before it can be used.

For investigational purposes, chloroform-extracted sera avoid expense in time and materials. This adapted procedure gives less critical results, but nevertheless appears to offer some discrimination between the quality of samples of both liquid and frozen egg. A more precise evaluation of the measure will be made in forthcoming papers of this series.

Acknowledgment

References

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.   Official and tentative methods of analysis.   5th ed. A.O.A.C., Washington, D.C.   1940.
2. BRYCE, W. A.   Unpublished data.
3. DAVIS, W. B.   Ind. Eng. Chem., Anal. Ed. 14 : 952-953.   1942.
4. FRYD, C..F. M. and HANSON, S. W. F.   J. Soc. Chem. Ind. 63 : 3-6.   1944.
5. HAWK, P. B., BERGEIM, O., OSER, B. I., and COLE, A. G.   Practical physiological chemistry. 11th ed. P. Blakiston's Son & Co., Inc., Philadelphia.   1937.
6. PEARCE, J. A. and THISTLE, M. W.   Can. J. Research, D, 20 : 276-282.   1942.
7. REID, M. and PEARCE, J. A.   Can. J. Research, F, 23 : 239-242.   1945.
8. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E.   Can. J. Research, D, 21 : 1-7.   1943.

DRIED WHOLE EGG POWDER

XIX. ACCELERATED STORAGE TESTS TO ASSESS THE EFFECT OF HEAT TREATMENT, MOISTURE CONTENT, AND MATERIALS ON THE QUALITY OF DRIED SUGAR-EGG MIXTURES

BY R. L. HAY AND JESSE A. PEARCE

DRIED WHOLE EGG POWDER

XIX. ACCELERATED STORAGE TESTS TO ASSESS THE EFFECT OF HEAT TREATMENT, MOISTURE CONTENT, AND MATERIALS ON THE QUALITY OF DRIED SUGAR-EGG MIXTURES[1]

BY R. L. HAY[2] AND JESSE A. PEARCE[2]

Abstract

Deterioration in quality was assessed by fluorescence, potassium chloride, pH, and foaming volume measurements.

Dried egg powder (moisture content, 2.8%), containing 33% sugar, and control samples of plain egg powder (moisture content, 3.9%) were stored at temperatures of 80°, 100°, 120°, and 140° F. for seven days. At 140° F. the addition of sugar inhibited the initial, but not the secondary, fluorescence development observed in the plain egg powder and retarded deterioration as assessed by other measurements. At temperatures of 120° F. and lower, the presence of sugar had a marked effect in retarding decrease in quality in egg powder as assessed by all quality tests used. Interpretation of the results in terms of commercial drying practices indicated that cooling shortly after drying was less important for sugar–egg powder than it was for plain egg powder.

Dried egg powder containing 33% sugar was adjusted to moisture levels of 1.4, 2.8, and 3.2% and stored at 80° and 120° F. for seven days. The rate of deterioration in quality of sugar–egg powder increased markedly with both moisture content and temperature. Egg powder containing 1.4% moisture maintained higher quality at both temperatures for a longer period than powders at either 2.8 or 3.2% moisture levels. It is recommended that sugar–egg powder be dried to the lowest moisture content compatible with the production of good quality powder, certainly to a moisture content of less than 2.8%, and preferably to 1.4%.

Loss in quality was less for sugar–egg powders (moisture content, approximately, 2.3%) prepared with granulated sugar than for those prepared with sucrose syrup, when stored at 80°, 100°, 120°, and 140° F. for seven days. In addition, powder made from fresh shell eggs was more desirable than powder prepared from frozen melange. It is recommended that sugar–egg powder be prepared from a mixture of sugar in granulated form and melange from fresh shell eggs.

Introduction

Eggs in powdered form have become a well known commodity during the war years. The production of egg powder in Canada during pre-war years was almost negligible but it is probable that the demand for this product will be increased during the post-war period. However, the extent to which this commodity competes successfully with other egg products during normal times will depend largely on how well its quality can be maintained, not only during production but also during subsequent handling and storage.

In a recent communication it was observed that the addition of sucrose to egg powder, prior to drying, was effective in delaying fluorescence development at temperatures of 118° F. and lower (9). Present indications are that this sugar–egg powder will find a ready peacetime market for baking and other trade purposes. .

Previous investigations have shown that temperature and moisture content were major factors in the preservation of dried foods.   Earlier studies showed that rapid cooling of plain egg powder to a temperature of 80° F. or less within three hours of leaving the drier was important if high quality were to be maintained (14, 15, 16).   Lowering the moisture content prolonged the storage life of this powder (7, 15) but even at the low level of 1.4% moisture, dried egg deteriorated in a relatively short time when stored at temperatures of 99° and 118° F. (13).   Moisture levels of 2% and less were believed desirable, but Canadian commercial drying operations did not permit the attainment of these lower levels.

Some sugar–egg powder is being produced in Canada at the present time. Current drying practice favours the production of sugar–egg from melange to which sucrose syrup rather than granulated sucrose has been added, although no comparison of the relative qualities of the final products has been made. Furthermore, no attempt has yet been made to compare the quality of sugar–egg powder prepared from shell eggs with that prepared from frozen melange.

Since sugar in egg had an inhibitory effect on fluorescence development, it was of interest to determine whether sugar–egg was as susceptible to heat deterioration as plain egg powder, to compare the effect of various moisture contents on the quality of sugar–egg powders, and to make some assessment of the function of sugar in this product.   This report also describes the effect of heat treatment on sugar-egg powders produced from the following: (*a*) shell eggs and sucrose syrup, (*b*) shell eggs and granulated sucrose, (*c*) frozen melange and sucrose syrup, and (*d*) frozen melange and granulated sucrose.

Comparisons of plain and sugar–egg powder in this study were made on the assumption that the moisture was almost entirely contained in the egg fraction of the sugar–egg.   Reference to equilibrium relative humidity data for egg powder showed that, at room temperature and about 3% moisture, the relative humidity over a sample of plain egg powder would be about 15% (5).   Reference to similar data (4) for sugar at 25° C. indicated that commercial sucrose (containing 0.05 to 0.2% invert sugar) would not begin to take up moisture until a relative humidity of about 70% was reached.   Even if inversion occurred during the drying process, it is unlikely that moisture would be elsewhere than in the egg powder, since sucrose containing 10% invert sugar does not begin to take up moisture until a relative humidity of about 25% is reached.

Materials and Methods

The powder containing 33% sugar (dry matter basis) and the plain egg powder used in the heat treatment study were obtained from the same commercial Canadian source.   These samples were tempered in the laboratory to a moisture content of approximately 4%, calculated on the basis of egg solids. The actual moisture content of the sugar–egg was 2.8%; the plain egg, 3.9%. The sugar–egg powder used in the moisture study was obtained from the same Canadian producer, and was tempered to moisture levels of 1.4, 2.8,

and 3.2%. Calculated on the basis of egg solids, the moisture contents were 2.1, 4.0, and 4.6% respectively. After the moisture adjustment had been completed samples of these powders were packed in tin-plate (air as headspace gas), stored at temperatures of 80°, 100°, 120°, and 140° F. and removed for analysis after one, two, five, six, and seven days.

The powders prepared from various mixtures (sugar content, 33%, dry weight basis) were obtained from another Canadian producer and adjusted in the laboratory to approximately the same moisture level (actual moisture content between 2.2 and 2.4%). Samples of these powders were also packed in tin-plate (air as headspace gas), stored at 80°, 100°, 120°, and 140° F., and removed for analysis after one, two, three, four, five, six, and seven days.

All powders were prepared on a cone type spray-drier. The choice of producers was a matter of convenience only. The drying temperatures were those believed most desirable for the respective pieces of equipment.

The quality of the powders was evaluated by measurement of fluorescence (8), potassium chloride value (12), pH (12), and foaming volume; the last was believed to provide a method of assessing the baking quality. The procedure for measuring the foaming volume of the plain egg samples was the same as that reported in an earlier study (11). For the sugar–egg samples, the foaming volume was assessed as follows: 40.5 gm. of the powder was mixed thoroughly with 75 ml. of distilled water. Best results were obtained by adding a small amount of the water to the powder, whipping the mixture manually into a thick homogeneous paste, and then pouring in the remainder of the water. The mixture was beaten at the highest speed in a "Mixmaster" for 10 min., and the volume of the foam measured in a graduate. Although the two methods used were not comparable, it was felt that relative differences occurring during storage would be apparent.

Results

The Effect of Heat Treatment

The relative effects of temperature and storage time on the quality of both types of egg powders were evaluated by means of analyses of variance. The significant results are shown graphically in Figs. 1*a* and 1*b*. Fluorescence values increased and potassium chloride values, pH values, and foaming volume decreased with both storage temperature and time.

At least two separate reactions may occur during the development of fluorescing materials in plain egg powder, since the previous curves have shown a definite break or irregularity during the formation of fluorescing substances (14). This has suggested the possible presence of an initial and a secondary reaction. In the present study, curves for the fluorescence development in materials containing no sugar showed evidence of two reactions, but the presence of sugar appeared to retard the initial reaction.

At temperatures of 120° F. and lower, sugar had a marked effect in reducing the rate at which fluorescence developed. Sugar–egg at 140° F. attained the
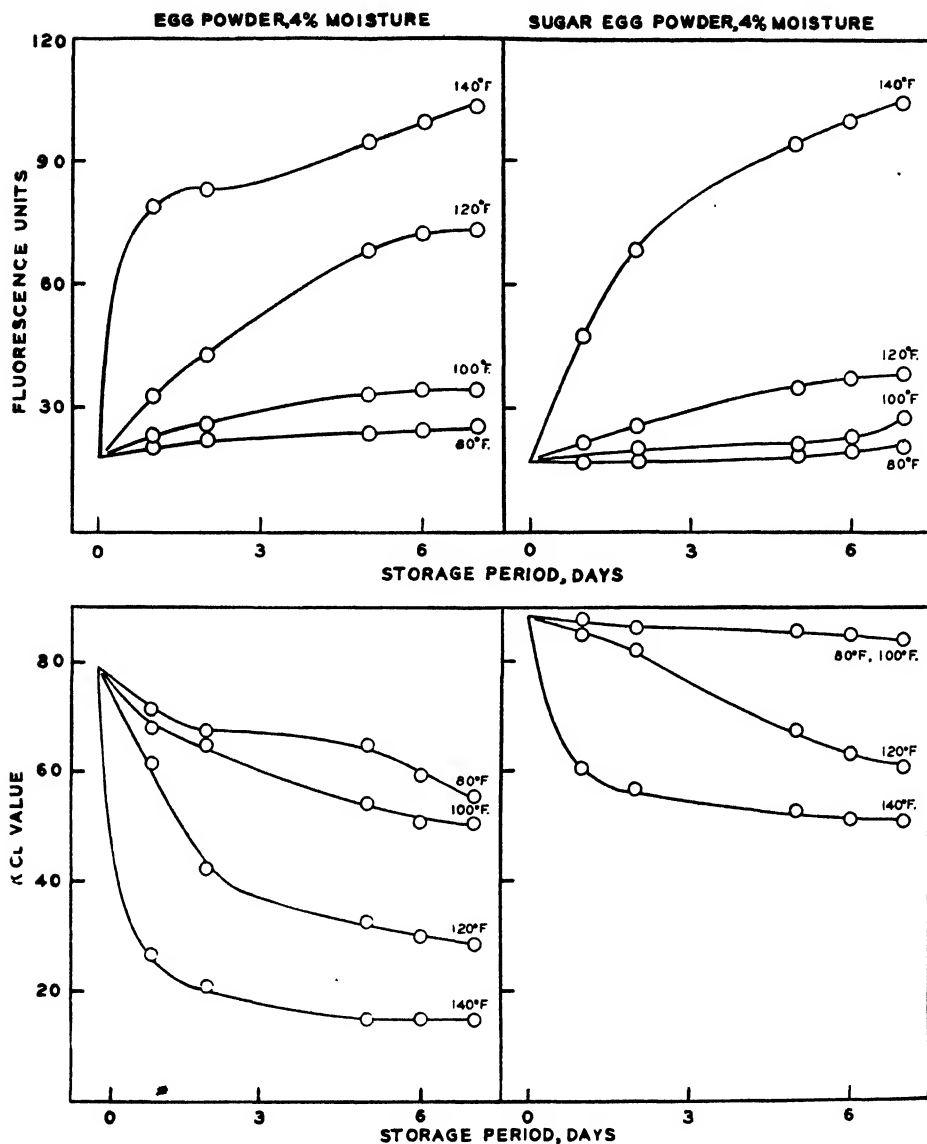
FIG. 1a.   Effect of heat treatment on fluorescence values and potassium chloride values of plain and sugar–egg powders with a moisture content of 4%, calculated on the basis of egg solids present.   (Actual moisture content of sugar–egg powder, 2.8%.)

same maximum value as plain egg, but the curve indicated retardation, elimination, or alteration of the first stage of the fluorescence reaction (Fig. 1a). At 140° F., the presence of sugar reduced the development of fluorescence by about one-half after storage for one day.

Earlier work had shown that sugar reduced fluorescence development during the first two weeks' storage at 118° F. and during eight weeks' storage
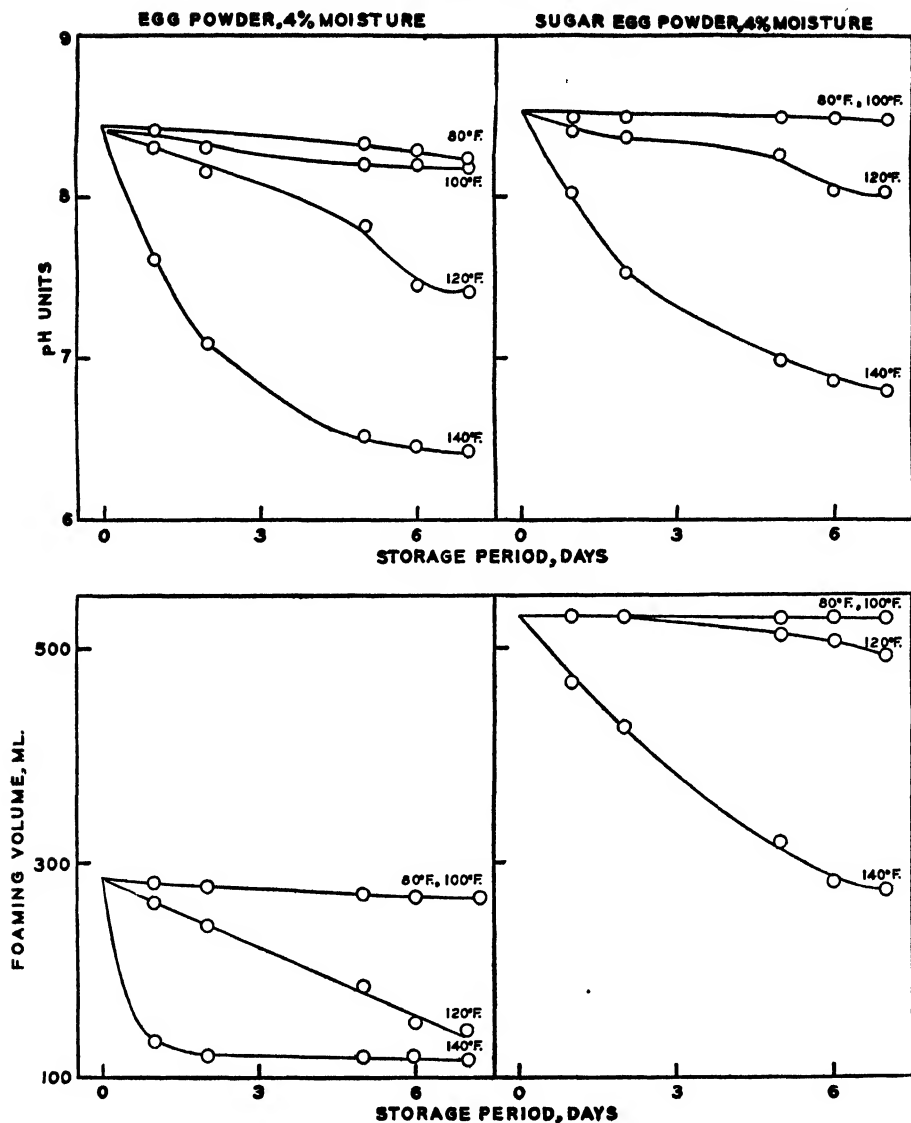
FIG. 1b.   *Effect of heat treatment on pH and foaming volume of plain and sugar–egg powders with a moisture content of 4%, calculated on the basis of egg solids present.   (Actual moisture content of sugar–egg powder, 2.8%).*

at lower temperatures (9).   The present study showed that sugar effectively retarded fluorescence development during seven days' storage at 80°, 100°, and 120° F.

The maximum fluorescence value allowable for Canadian Grade *A* plain egg powder has been established at 24, and that for Grade *B* at 57 (3).   Prime quality sugar–egg powder stored at 80° F. remained at a lower fluorescence level than that of plain egg during the entire period of seven days (Fig. 1a).

At 100° F., high quality was maintained in the sugar–egg five days longer than in the non-sugar powder. Best quality sugar–egg powder stored at 120° F. decreased to Grade *B* quality in 48 hr., while only about eight hours were required for a similar deterioration in plain egg powders. At 140° F., sugar appeared to have an inhibitory effect sufficient to delay deterioration for several hours.

Fluorescence development as depicted in Fig. 1*a* revealed that sugar added to egg powder before drying provided a beneficial effect on quality. Since sugar–egg powder was less susceptible to heat treatment it appeared that cooling to 80° F. shortly after drying would not be as important for sugar–egg as it is for plain egg.

The solubility of plain egg powders in a 10% potassium chloride solution decreased when either temperature or storage time was increased, and this was believed due to thermal decomposition of a fat–protein complex and denaturation of the egg protein (14).

In this study (Fig. 1*a*), the solubility of plain egg decreased during storage at all temperatures, the most marked change occurring in the first two days at 140° and 120° F. Subsequent changes at these temperatures were relatively slow; this indicated that the denaturation processes may have been approaching completion. The presence of sugar in egg powder appeared to provide the most protection at 80° and 100° F., and a comparison of the curves for sugar–egg and plain egg powders at 120° F. also showed evidence of some preservative action.

Correcting the potassium chloride values of sugar–egg powder for the presence of added sugar indicated that about 80% of the egg solids were soluble, which is about the same quantity usually soluble in fresh plain egg powder. After two days at 140° F., plain egg powder was only 20% soluble, but the egg fraction of sugar–egg powder was still about 40% soluble. This indicated that the presence of sugar in some way retarded or impeded thermal decomposition of the fat–protein complex or protein denaturation.

It has been reported that a decrease in pH accompanied quality deterioration in egg powders (12, 14). Further evidence of this is apparent in Fig. 1*b*. The pH of the plain egg powder decreased at all temperatures, with the greatest reduction occurring during the first five days at 140° F. The stabilizing effect of sugar as indicated by an almost constant pH was most evident at temperatures of 80° and 100° F. Sugar had a slightly beneficial effect at 120° F., but less at 140° F. These observations agreed with, and supported, the results obtained from the more sensitive fluorescence and potassium chloride tests.

For the plain egg powder the decrease in foaming volume at both 80° and 100° F. was relatively small, and of approximately the same order of magnitude. At 120° F. the decrease in this attribute was greater than at the lower temperatures, but the greatest change occurred in the plain egg during the first 24 hr. at 140° F. The subsequent changes at the high temperature were extremely slow; this indicated that the components responsible for the foaming property had been altered rapidly.

Although the techniques for determining foaming volume differed for plain and sugar–egg, and a marked increase in foaming volume is known to result from the addition of sugar to egg powder prior to drying, it was felt that the relative changes noted here were comparable (1). At 80° and 100° F. the foaming volumes of sugar–egg remained approximately equal to the initial value during the entire storage period. At 120° F. the foaming volume of sugar–egg powder decreased only 46 ml. after seven days in storage. Under the same conditions, the foaming volume of the plain egg powder decreased 145 ml. Thus, sugar had marked effect in maintaining high foaming volumes at 120° F. At 140° F. total loss of the components responsible for the foaming quality appeared to have been postponed several days owing to the presence of sugar.
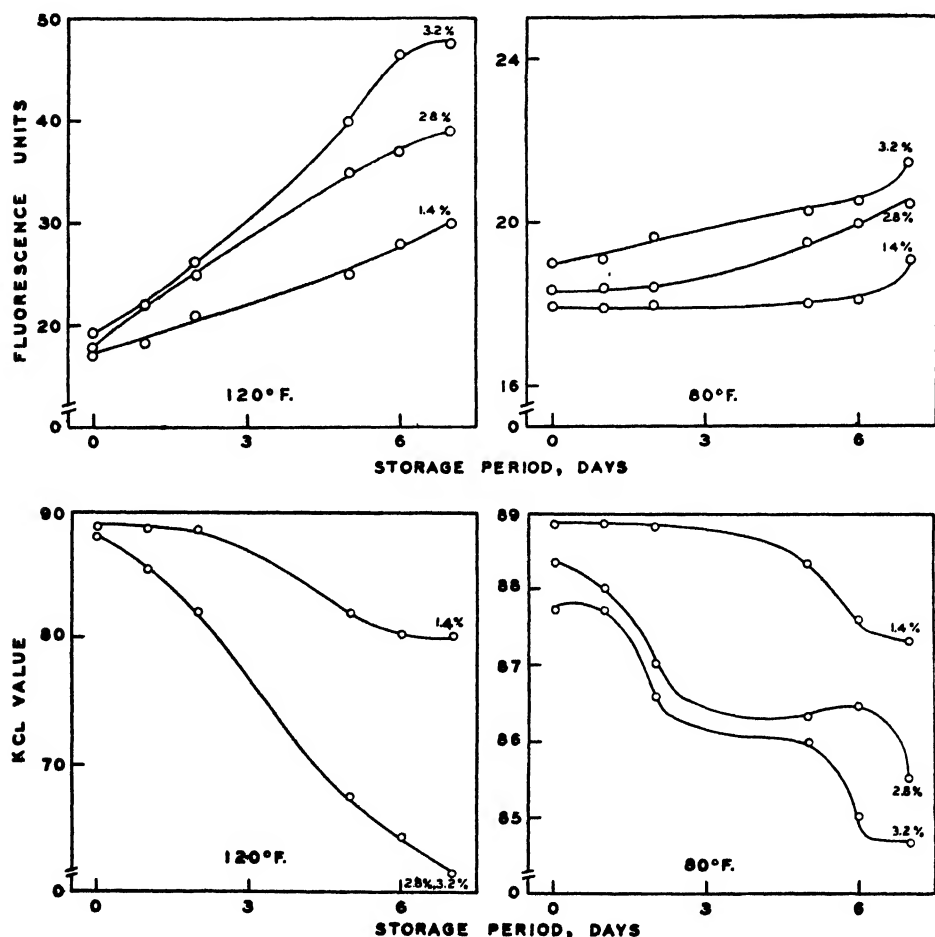
FIG. 2a. *Effect of heat treatment on fluorescence values and potassium chloride values of sugar–egg powder having actual moisture contents of 1.4, 2.8, and 3.2%.*

The Effect of Moisture Content

The effect of moisture content on the quality of sugar–egg powders was also evaluated by means of analyses of variance, and the significant results are shown graphically in Figs. 2*a* and 2*b*.   The general effects of temperature and storage time noted in the heat treatment study were again observed in this study.   Powder containing 1.4% moisture was better initially, except for foaming volume, and remained superior to the other powders during storage. The 3.2% powder produced the highest initial foaming volume but after the sixth day in storage deteriorated to a level below that of the lower moisture powders.

The rate at which the fluorescence value increased was roughly proportional to the moisture content.   At 80° F., egg powder with a 3.2% moisture
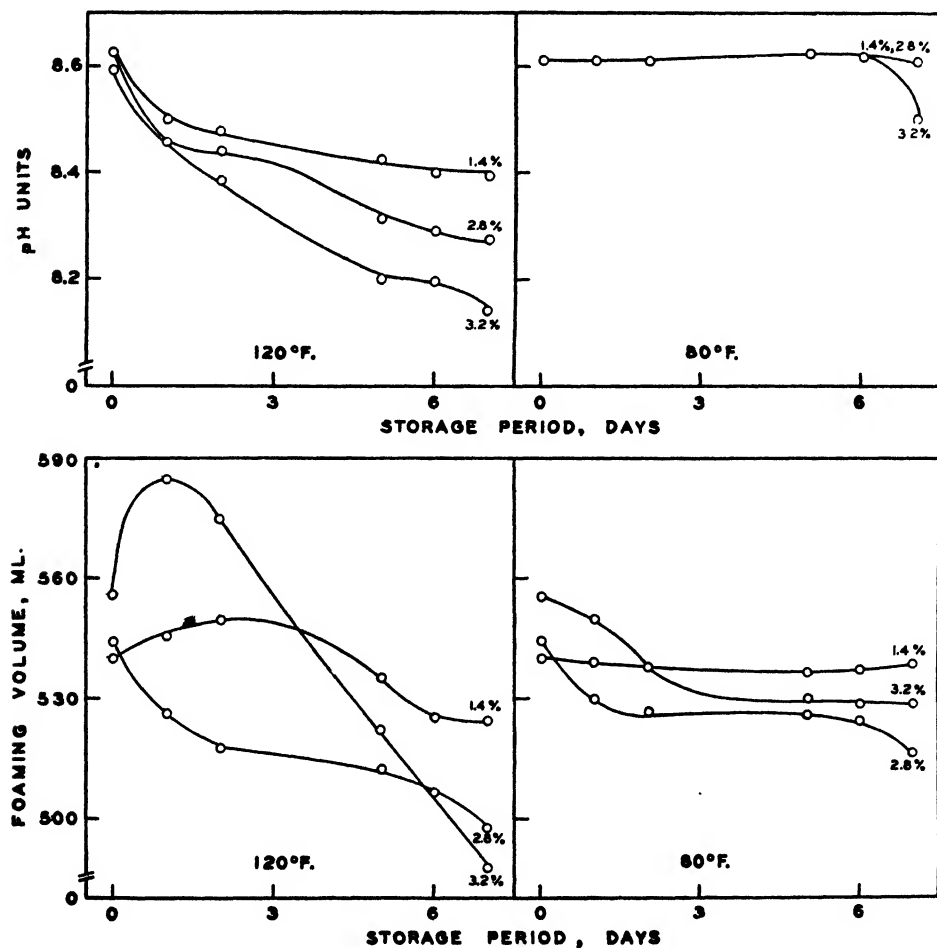
FIG. 2*b*.   *Effect of heat treatment on pH and foaming volume of sugar–egg powder having actual moisture contents of 1.4, 2.8, and 3.2%.*

content remained at a higher fluorescence level than either the 1.4 or 2.8% powders throughout the storage period of one week. At 120° F., powder containing 1.4% moisture had an initial fluorescence value of 17 and this increased linearly to a final value of 30 after the seventh day of storage. The 2.8 and 3.2% powders deteriorated at approximately the same rate during the first few days but attained final fluorescence values of 39 and 48, respectively. The 2.8 and 3.2% powders had attained a fluorescence value of 24, the maximum value allowable for Canadian Grade *A* egg powder (3), after two days of storage at 120° F., while five days were required for a similar deterioration in the 1.4% powder; this indicated that drying sugar–egg to a low moisture content might extend the life of the product about two and one-half times.

Egg powder containing 1.4% moisture and stored at 80° F. for seven days had a higher solubility than either the 2.8 or 3.2% powders. The solubility of the egg powders at all three moisture levels decreased more rapidly during storage at 120° F. than during storage at 80° F., with the most marked decreases occurring in powders with moisture contents of 2.8 and 3.2%. A comparison of the curves (Fig. 2a) for powders stored at 120° F. showed that loss in solubility in the 1.4% powder was approximately one-third that in the 2.8 and 3.2% powders after storage for one week.

Sugar–egg powders containing 1.4% moisture maintained higher pH values for a longer period than those powders at either 2.8 or 3.2% moisture levels (Fig. 2b). At 120° F., the decrease in pH was most rapid for the powder with 3.2% moisture. The powder with 1.4% moisture maintained the highest pH level during the entire storage period.

These results of foaming volume measurements on the stored powders were slightly different from those shown by the previous measurements. During the first few days 3.2% moisture appeared to be most desirable but after seven days the foaming volumes of this powder had decreased rapidly to a level below that of the 1.4 and 2.8% powders. The curves indicated that this initial beneficial effect was of short duration and that after an extended storage period the powder containing the lowest moisture would be most desirable. Extended studies on the change in foaming volumes of sugar–egg powders are currently under investigation in these laboratories.

Effect of Materials Used

The effects of temperature, storage time, method of adding sugar, and prior condition of melange, on quality of the sugar–egg powder were also evaluated by means of analyses of variance.   Although initially the powders did not differ in quality, every test used on the stored samples showed significant differences to result from the different materials used (Tables I and II). Differential behaviour of significance is shown in Figs. 3 and 4. Both the tables and the figures contain mean values of each variable, as calculated over all others for the whole experiment.

TABLE I

EFFECT OF HEAT TREATMENT ON SUGAR–EGG POWDERS PREPARED FROM SHELL EGGS AND
FROM FROZEN MELANGE

| Testing method | Mean value for powder prepared from | | | |
| | Shell eggs | | Frozen melange | |
	Initially	After storage*	Initially	After storage*
Fluorescence value	20.0	30.2	22.2	27.4
Potassium chloride value	86.4	79.3	87.3	77.8
Foaming volume	632	611	602	568
pH value	8.64	8.42	8.57	8.36

* *Averaged over all storage times and temperatures.*

TABLE II

EFFECT OF HEAT TREATMENT ON SUGAR–EGG POWDERS PREPARED WITH SUGAR AND WITH SYRUP

| Testing method | Mean value of powder prepared with | | | |
| | Granulated sugar | | Syrup | |
	Initially	After storage*	Initially	After storage*
Fluorescence value	20.5	28.2	21.7	29.6
Potassium chloride value	87.3	79.2	86.0	77.9
Foaming volume	638	623	600	556
pH value	8.64	8.44	8.56	8.34

* *Averaged over all storage times and temperatures.*

The rate of deterioration in all powders increased with both temperature and storage time (Fig. 3), and the trends observed were similar to those noted in the earlier study (Figs 1a and 1b). Comparison of the curves in Fig. 3 with those in Figs. 1a and 1b shows the beneficial effect of a reduction of 0.5% in moisture content.

The initial measurements showed small differences between the various samples of the dried product, and all but the fluorescence measurements on the stored powders supported the evidence that shell egg produced a better dried material than frozen melange (Table I). However, another investigation in these laboratories has shown that freezing reduces the fluorescence of liquid egg (10) and also reduces the intensity of the light given off by fluorescing materials isolated from egg powder (2). Therefore, the low fluorescence values for stored powders prepared from frozen material do not necessarily indicate high quality. The superiority of shell egg over frozen egg was shown most markedly by potassium chloride and foaming volume measure-
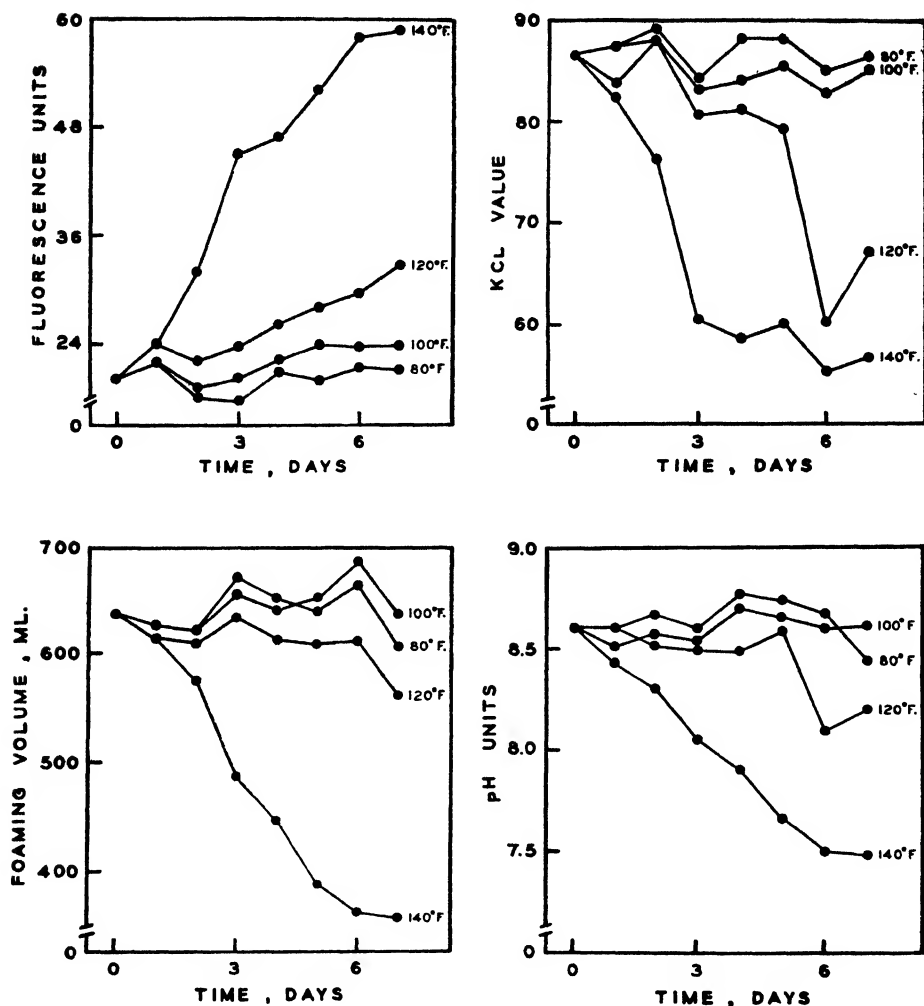
FIG. 3. *Average effect of heat treatment on fluorescence values, potassium chloride values, pH, and foaming volume of sugar–egg powder prepared from shell and frozen eggs using sucrose crystals and sucrose syrup. Actual moisture content of these samples was about 2.3%. Comparison with Fig. 1a indicates the improvement resulting from 0.5% reduction in moisture content.*

ments. Freezing the melange apparently made some of the soluble constituents, possibly those responsible for the aerating properties in egg, less stable. This is receiving further consideration in these laboratories.

Sugar–egg powder prepared by adding sugar appeared slightly better initially than the product prepared from syrup, and when the powders were stored these differences became significant as assessed by all measurements (Table II). The initial 38 ml. difference in foaming volume values was considered most important, especially as sugar–egg is used only for baking. In addition, this difference was accentuated by the storage treatment.
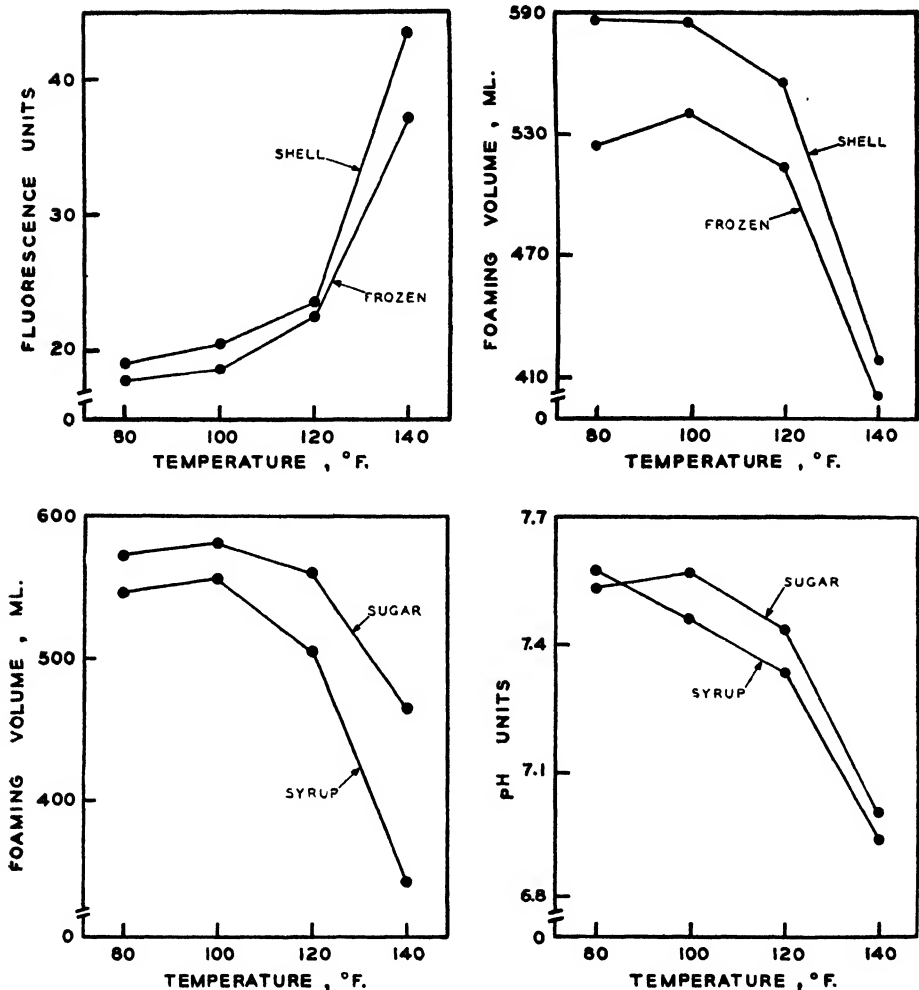
FIG. 4. *Average effect of temperature on some of the quality measurements applied to sugar–egg powder prepared from shell and frozen eggs, using sucrose crystals and sucrose syrup. Actual moisture content of these samples was about 2.3%.*

The effect of temperature on the fluorescence and foaming volume measurements of sugar–egg powders made from shell egg and from frozen melange, is presented graphically in Fig. 4. Powder produced from frozen melange had lower fluorescence values at all storage temperatures studied. At 80°, 100°, and 120° F. increases in fluorescence were slight and approximately parallel, while at 140° F. both showed very rapid increases in fluorescence development. The divergence of the curves indicated that powder from frozen melange developed fluorescing substances more slowly as the storage temperature was increased from 120° to 140° F. The foaming volume curves showed the pronounced superiority of shell eggs over frozen melange as a

component of sugar–egg powder when subjected to storage temperatures of 80°, 100°, and 120° F.

Sugar–egg powder prepared with syrup and stored at elevated temperatures was more susceptible to deterioration than the product made with granulated sugar (Fig. 4). Although the foaming volume of the latter product, after storage at 80° and 100° F. was better by only about 35 ml., this difference progressively increased to approximately 125 ml. at 140° F. Since a decrease in pH also accompanies quality deterioration it is evident from the pH curves that egg powder prepared with syrup deteriorated more rapidly than that prepared with sugar, when the powders were stored at 100° and 120° F. At 80° and 140° F. the difference between mean pH values, although favouring the powder prepared with sugar, was very small.

Discussion

The results of the heat treatment study show that the addition of sugar to egg prior to drying helps to maintain those qualities desirable for baking. Cooling after drying was less important for sugar–egg powder than it was for plain egg. Nevertheless, it is believed desirable to maintain the cooling practices in current use by industry.

The results of the moisture study indicated that the water content of sugar–egg powder should be below 2.8% and preferably about 1.4% if quality comparable to that of fresh egg powder is to be maintained during storage.

For comparison of the effect of moisture content in sugar–egg powder there was no need to correct fluorescence and potassium chloride values for the presence of added sugar, since all measures were relative. However, to compare plain and sugar–egg powders some adjustment was necessary. This has been done for the potassium chloride values shown in Fig. 5. Since the increase in fluorescence due to the caramelization of the sugar was difficult to evaluate, no correction was made for the fluorescence values of sugar–egg powder. However, it was believed that even if this correction were made the fluorescence curves in this figure would be only slightly altered and the conclusions would be much the same.

Since the sugar in the dried product can be assumed to have a negligible moisture content, all the moisture in sugar–egg powder is probably in the egg fraction. It is possible that the moisture may be equally distributed throughout both sugar and egg fractions, and the beneficial effects attributable to sugar may be due to the ability to dry to a low moisture content, thereby reducing the moisture in the egg fraction. The solid circles and squares shown in Fig. 5 represent an interpolated value for plain egg powder at 3.5% moisture and 120° F. from data previously reported (14) and an actual value for plain egg powder at 3.9% moisture and 120° F. from the heat treatment study, determined after two days' storage. The fluorescence increment and potassium chloride decrement of sugar–egg powders indicated that this product when stored at 120° F. changed by about the same amount as plain egg

powder stored at 110° F. for the same time and certainly much less than can be expected for plain egg powder stored at 120° F. This comparison indicates that sugar exerts a pronounced beneficial effect separate from any suggestion of low moisture content in the egg fraction attributable to distribution of the moisture between the egg solids and the sugar solids.
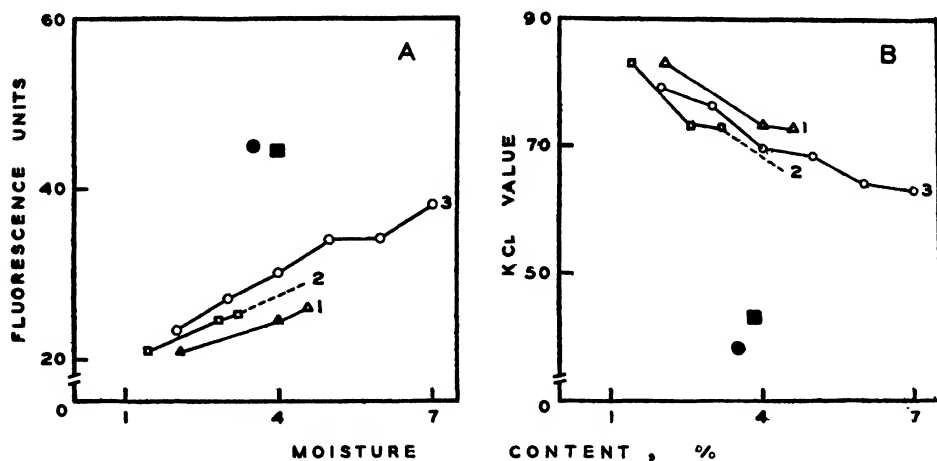

Fɪɢ. 5. *Effect of moisture content and added sugar on the fluorescence and potassium chloride values of dried egg powder after two days' storage. Curve 1—sugar-egg stored at 120° F.; moisture content calculated on basis of egg solids. Curve 2—sugar-egg (actual moisture content) stored at 120° F. Curve 3—plain egg powder (actual moisture content) stored at 110° F. (15).* ■ *Plain egg powder, 3.9% moisture, from heat treatment study, storage temperature 120° F.* ● *Plain egg powder, 3.5% moisture, value interpolated for 120° F. from data previously reported (14).*

Some physical or chemical combination may occur between sugar and the components of the egg, and provide protection to the product not only during the drying process but during subsequent handling. Although the nature of this combination is at present in doubt, the practical aspects are of significance, and are receiving further attention in these laboratories.

The results of the study on powder prepared in different ways showed that the storage life of sugar-egg powder was improved by adding granulated sugar instead of syrup to the liquid egg prior to drying. This may be explained on the basis of drying operations. The mixture made with syrup had a higher moisture content than the mixture prepared with solid sugar. Therefore, to obtain the same production rate in terms of solids requires a higher drying temperature (4, 12, 17), and corresponding deterioration in the product would be expected. To obtain drying at the same inlet and outlet temperatures necessitates reduction in melange input. If it is assumed that the liquid particles from the egg-syrup mixture are identical in size with the liquid particles from egg-sugar mixture, the dried particles would be smaller, settle more slowly and, as a result, may be exposed to a longer period of heating in the drier, thus causing reduction in quality of the product.

This study also showed that melange from fresh shell eggs produces a sugar–egg powder superior to that from frozen melange.   Egg melange is a colloidal complex containing in solution proteins, fats, a trace of sugar, lecithin, and about 1% of salts.   It is known that colloids when frozen do not normally recover their original state on thawing, a phenomenon that is probably due to precipitation or coagulation of the proteins during freezing. It has been observed that egg yolk, frozen, stored below $-6°$ C. for a reasonable time and then thawed, lost its fluidity and passed into a viscous condition with a reduced volume (6).   A similar treatment caused the white to separate into liquid and viscous parts with the former increasing at the expense of the latter by an amount depending upon the temperature and storage time. The rate of freezing and thawing of the egg has also been found to affect the composition of the resulting melange (6).   These changes may be responsible, in part at least, for the differences in behaviour of powders prepared from shell egg and from frozen melange.

Acknowledgments

References

1. BROOKS, J. and HAWTHORNE, J. R.   J. Soc. Chem. Ind. 62 : 165-167.   1943.
2. BRYCE, W. A.   Unpublished data.
3. CANADA DEPARTMENT OF AGRICULTURE.   Special products board requirements No. 6.
      Ottawa.   28 Feb. 1944.
4. DITTMAR, J. H.   Ind. Eng. Chem. 27 : 333-335.   1935.
5. GANE, R.   J. Soc. Chem. Ind. 62 : 185-187.   1943.
6. MORAN, T. and PIQUE, J.   The storage of eggs.   Dept. Sci. Ind. Research, Food Investi-
      gations.   Special Rept. No. 26.   H.M. Stationery Office, London, England.   1926.
7. PEARCE, J. A., REID, M., and COOK, W. H.   Can. J. Research, F, 24 : 39-46.   1946.
8. PEARCE, J. A., THISTLE, M. W., and REID, M.   Can. J. Research, D, 21 : 341-347.   1943.
9. PEARCE, J. A., WOODCOCK, A. H., and GIBBONS, N. E.   Can. J. Research, F, 22 : 34-38.
      1944.
10. REID, M.   Can. J. Research, F, 24 : 130-135.   1946.
11. REID, M. and PEARCE, J. A.   Can. J. Research, F, 23 : 239-242.   1945.
12. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E.   Can. J. Research, D, 21 : 1-7.   1943.
13. THISTLE, M. W., WHITE, W. H., REID, M., and WOODCOCK, A. H.   Can. J. Research, F,
      22 : 80-86.   1944.
14. WHITE, W. H. and THISTLE, M. W.   Can. J. Research, D, 21 : 194-202.   1943.
15. WHITE, W. H. and THISTLE, M. W.   Can. J. Research, D, 21 : 211-222.   1943.
16. WHITE, W. H., THISTLE, M. W., and REID, M.   Can. J. Research, D, 21 : 271-276.   1943.
17. WOODCOCK, A. H. and REID, M.   Can. J. Research, D, 21 : 389-393.   1943.

LIQUID AND FROZEN EGG

II. METHODS OF DETERMINING SOLIDS CONTENT OF LIQUID AND FROZEN EGG[1]

By C. G. Lavers[2] and Jesse A. Pearce[3]

Abstract

Solids content of liquid egg prepared from shell eggs having different histories was measured by the official A.O.A.C. vacuum oven method and compared with measurements of specific gravity and refractive index. Specific gravity measurements were the least satisfactory, but may provide a rough check on solids content. Refractive index measurements following treatment with ammonium hydroxide were a more satisfactory measure of solids content than the same measurement on liquid egg after treatment with trypsin solution.

Relations between the solids content of defrosted frozen egg and unfrozen liquid egg and refractive index, as determined with a Zeiss sugar refractometer and with a hand sugar refractometer, were calculated for the method involving the addition of ammonium hydroxide. The relation, solids–refractive-index, for unfrozen liquid egg differed from the relation for frozen egg. However, the method provided a rapid, convenient, and accurate means for determining solids content.

Introduction

In recent years dried egg production has increased markedly. Since drying capacity is limited, all the eggs produced during the peak laying season cannot be dried or used immediately. Much of this egg is frozen and held in storage for subsequent drying, or for use as thawed liquid egg by bakers and others. One problem of importance in drying and freezing liquid egg is that of a rapid test for the solids content. The use of refractive indices for this purpose has been described (4). The present paper compares two of these refractometric methods (2, 4) and a hydrometric method with the official A.O.A.C. vacuum oven method (1, p. 308).

Materials and Methods

Preliminary experiments indicated that liquids prepared from eggs having different histories differed in their initial solids content, and in their behaviour on dilution. Therefore, dilutions of liquid prepared from eggs with a variety of histories were used in this study. To compare the various methods, the following eggs were used: currently available Grade *A* large summer eggs; Grade *A* large summer eggs obtained within one day of laying; Grade *A* large spring eggs, stored at 0° C. (32° F.) for three months (all grades refer to condition of eggs before storage); Grade *A* large spring eggs held in commercial storage for three months; Grade *A* large spring eggs stored at 0° C. for three months followed by two weeks at 21.1° C. (70° F.); currently available

---

[2] *Chemical Engineer, Food Investigations.*

[3] *Biochemist, Food Investigations.*

Grade *A* pullet summer eggs; and Grade *C* summer eggs. To obtain a more representative sample, eggs of the following grades were added to the above in determining the final relation between refractive index and solids content; Grade *A* large early spring eggs; Grade *A* pullet spring eggs; Grade *C* spring eggs. In addition, frozen egg of five different grades, ranging from top quality to mouldy, was used in the investigation.

All samples of both liquid and defrosted frozen egg were prepared for analysis by mixing with a "Mixmaster" at low speed. The official A.O.A.C. vacuum oven method for determining solids content (1, p. 308) was used as a standard against which the various methods were compared.

Specific gravities were determined using a hydrometer having a range of 1.000 to 1.070 (60°/60° F.). In making the determination, it was found necessary first to remove the foam from the surface of the liquid egg, allow the hydrometer to sink gently in the liquid to a steady position, then push it down one or two scale divisions and let it rise to an equilibrium position. Through a series of 35 determinations at temperatures ranging from 10° to 35° C. (50° to 95° F.) the temperature gradient was observed to be -0.00032 specific gravity units per °C. rise in temperature (-0.00018 units per °F.). Using this figure, all determinations were corrected to give specific gravity at 15.6° C. (60° F.).

The two refractometric methods used have been described (2, 4). One of these depended on an enzymatic (trypsin) digestion (4), the other on the use of an electrolyte, 28 to 29% reagent grade ammonium hydroxide (2). The former method involved the use of a trypsin solution (trypsin, 500 gm.; water, 770 ml.; and 0.25 *N* sodium hydroxide, 800 ml.), having a refractive index of 1.377 at 30° C. To 10 gm. of whole egg, 1.8 ml. of this solution was added, followed by thorough mixing. Several drops of the mixture were placed on the prisms of the refractometer and after 30 sec. the refractive index was read. The latter method required the addition of 10 drops of ammonium hydroxide to 20 ml. of the egg sample, mixing, and determination of the refractive index as before. All refractive indices were determined at 30° C. $\pm$ 0.5° C.

For the major portion of this work a Zeiss sugar refractometer was used. In addition, the use of a hand refractometer was considered as a more convenient method for plant purposes. This instrument was a Bausch and Lomb hand sugar refractometer, reading from 0 to 60% sugar.

Results

Comparison of Methods

Refractometric and hydrometric measurements showed a high correlation with solids content as determined by the official A.O.A.C. vacuum oven method. Equations expressing the relation between these rapid determinations and per cent solids in liquid egg, and estimations of the error that would be involved in using them to predict solids content, are given in Table I. While the equations relating solids content in any one type of egg to the other measurements differed somewhat in slope (Figs. 1, 2, and 3), these differences

TABLE I

EQUATIONS, WITH ERRORS OF PREDICTION, RELATING PER CENT SOLIDS IN LIQUID EGG (y) TO
VALUES BY RAPID METHODS (x)

Method	Equation	Error of prediction, % solids
Specific gravity (hydrometric)	$y = 793.02\,x - 794.77$	± 1.3
Refractive index (enzymatic)	$y = 678.04\,x - 907.94$	± 0.66
Refractive index (electrolytic)	$y = 574.75\,x - 766.02$	± 0.42
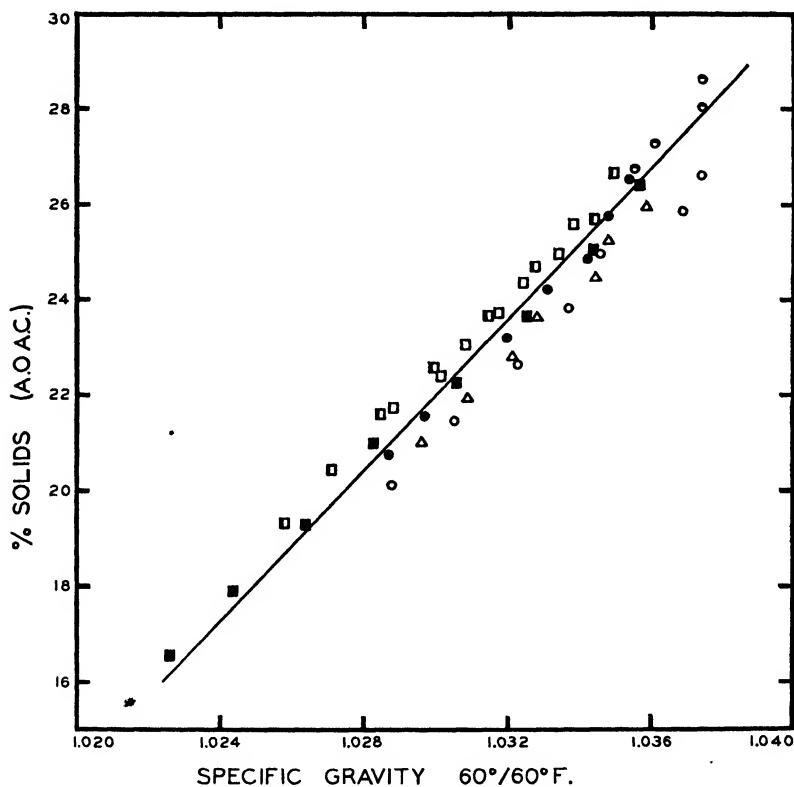
FIG. 1. *Relation between solids content of liquid egg and specific gravity.*

Key

☐ *Grade A large summer eggs obtained within one day of laying.*
■ *Currently available Grade A large summer eggs.*
◧ *Currently available Grade A pullet summer eggs.*
○ *Grade A large spring eggs held three months at 0° C. (32° F.).*
● *Grade A large spring eggs held three months in commercial storage.*
◒ *Grade A large spring eggs held three months at 0° C., then two weeks at 21.1° C. (70.0° F.).*
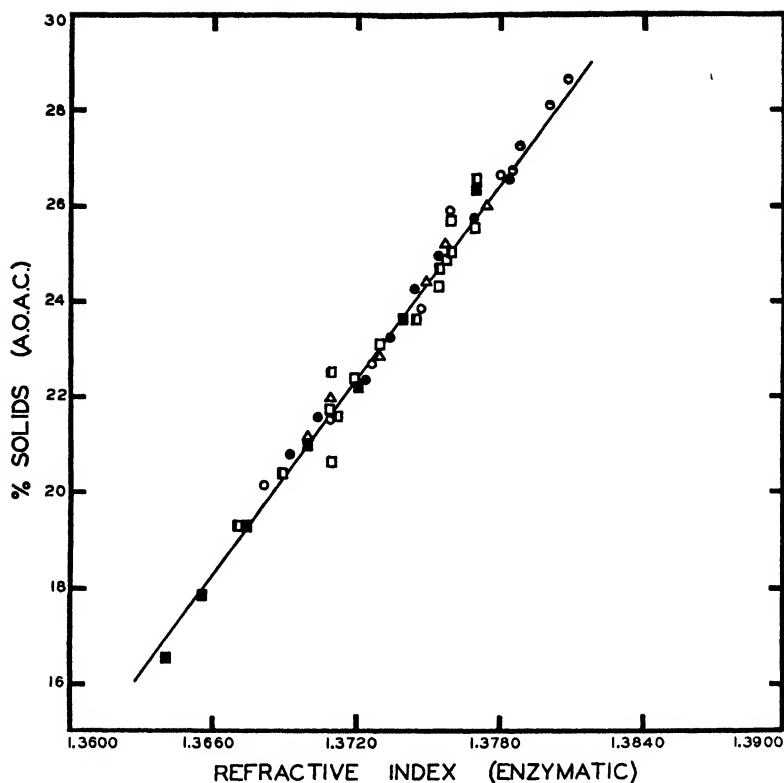△ *Grade C summer eggs.*

FIG. 2. *Relation between solids content of liquid egg and refractive-index after treatment with trypsin (Key in caption for Fig. 1.).*

did not hinder combination of the data to establish the mean relations shown in the figures.   These equations are based on equal numbers of determinations performed on identical samples, and are of value chiefly for comparative purposes.   Best estimate equations recommended for predicting solids content from refractive index (electrolytic method) are discussed later.

The error in determining solids content by the specific gravity method was of sufficient magnitude to preclude its use, except as a rough check.   It was evident that differences resulting from previous history exerted a pronounced influence on the relation obtained.   This method was unsatisfactory for frozen egg owing to the lack of homogeneity in the thawed material.   Attempts at homogenizing the melted egg resulted in the incorporation of air into the product, which did not pass off even on long standing, thereby preventing accurate determinations.

Both refractometric methods were relatively satisfactory (Table I and Figs. 2 and 3).   Refractive index measurements on liquid egg after treatment with ammonium hydroxide was the most satisfactory of the methods investigated.   The accuracy of prediction was somewhat better.   The use of ammonium hydroxide rather than the trypsin solution was simpler from the
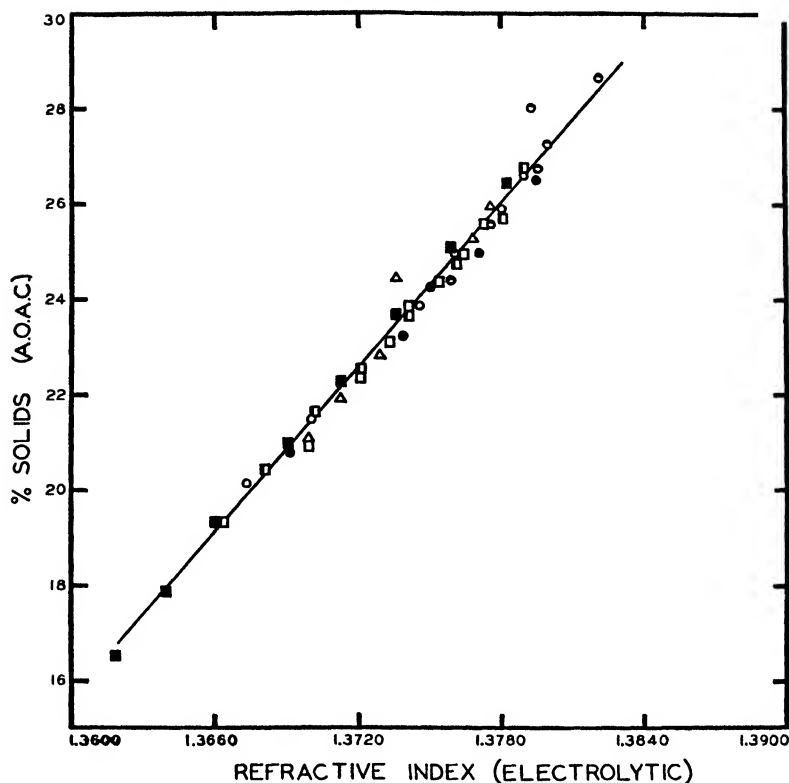
FIG. 3.   *Relation between solids content of liquid egg and refractive index after treatment with ammonium hydroxide.   (Key in caption for Fig. 1.)*

point of view of laboratory technique, and in addition gave a more easily readable field in the refractometer.

Before putting any of the above determinations into practical use as a standard method of measuring solids in liquid egg, it would be advisable to evaluate differences of technique existing between laboratories.   The calibration curves shown in Figs. 2 and 3 are approximately 0.5 and 1% lower respectively than those previously recorded (2, 4).   This disagreement may be attributable to the different methods of obtaining liquid egg of varying solids content.

Best Estimate Equations

Since the initial comparison showed the refractometric method involving the use of ammonium hydroxide to be the best of those considered, it was used in all subsequent work.   Further measurements were made using this method to include determinations on eggs produced over the major portion of the Canadian laying season in computing the final prediction equation. The method was also used on thawed frozen egg.   Equations, and errors of prediction, for both liquid and melted frozen egg are given in Table II.   These relations are shown graphically in Fig. 4.   It will be noted that the equations

TABLE II

EQUATIONS, WITH ERRORS OF PREDICTION, RELATING PER CENT SOLIDS IN
EGG (y) TO REFRACTIVE INDEX, ELECTROLYTIC METHOD (x)

Kind of egg	Equation	Error of prediction, % solids
Liquid	$y = 593.53\,x - 791.68$	$\pm\ 0.76$
Frozen	$y = 587.08\,x - 781.56$	$\pm\ 0.48$
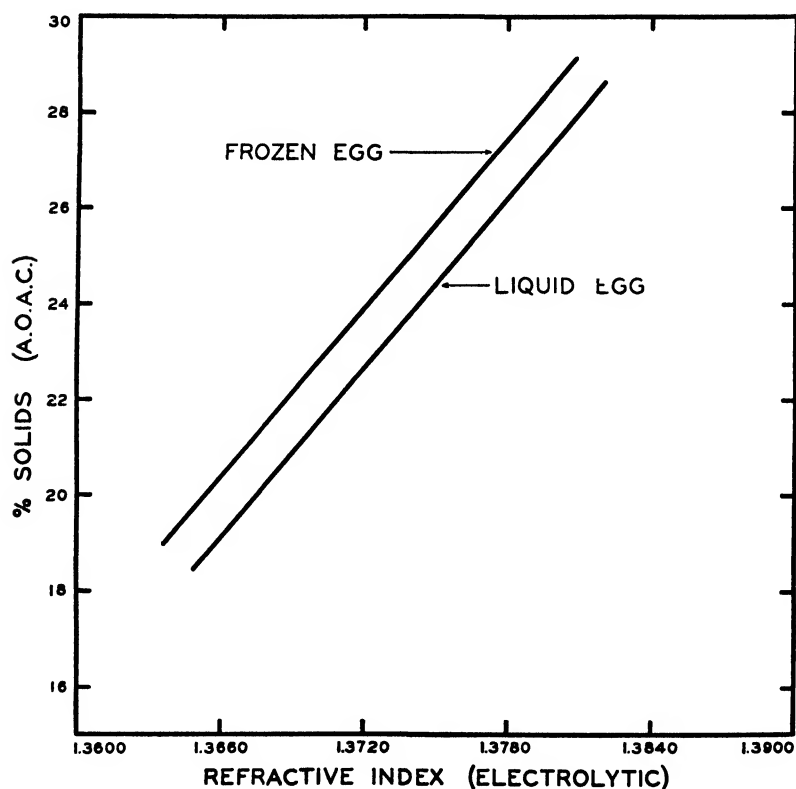
FIG. 4. *Relations between solids content of egg (liquid and frozen) and refractive index after treatment with ammonium hydroxide (as calculated from the best estimate equations).*

for liquid and frozen egg have approximately the same slope, but quite different intercepts, and so are not interchangeable.

This method of determining solids in egg is rapid, simple, and sufficiently accurate for industrial purposes.

Determinations Using a Hand Refractometer

The instrument used in the refractometric work so far described was not portable, and required a constant temperature bath. For routine inspection

a portable instrument would be very advantageous. For this reason, consideration was given to an easily obtainable, relatively inexpensive hand refractometer, designed to determine sugar concentration directly. No method of temperature control was provided for this hand instrument, but a correction thermometer reading in per cent solids for sugar was mounted on the side of the refractometer.

For a series of 18 readings at temperatures ranging from 19.0° to 32.0° C. (66.2° to 89.6° F.) the change of refractive index of liquid egg with temperature was observed to be −0.00012 refractive index units per °C. rise in temperature, (−0.00007 units per °F.). The corresponding figure for sugar solutions, as calculated from available data (3), is approximately −0.00014 (−0.00008). Since these two figures are nearly the same, the temperature correction given for sugar may be applied directly to egg.

Since the relation between refractive index and per cent sugar of sugar solutions is known (3), and that between refractive index (electrolytic method) and per cent egg solids is given in Table II, it was possible to calculate the equations given for converting readings on the hand sugar refractometer to per cent egg solids (Table III). Although these equations could be calculated it was necessary to know the error of prediction involved when using this hand instrument. To evaluate this, a series of 23 determinations was made on identical samples using both the hand and Zeiss sugar refractometers. This gave a direct comparison of the error involved in using each instrument. With this knowledge it was possible to calculate, by simple ratio, the error of prediction involved when using the hand instrument and the prediction equations given in Table III, because these equations were based on the same determinations as those given in Table II.

TABLE III

EQUATIONS, WITH ERRORS OF PREDICTION, RELATING PER CENT SOLIDS IN
EGG (y) TO READINGS ON A HAND REFRACTOMETER (x)

Kind of egg	Equation	Error of prediction, % solids
Liquid	$y = 1.024 x - 3.59$	± 0.56
Frozen	$y = 1.016 x - 2.14$	± 0.35

The procedure for determining per cent solids with the hand refractometer would then be as follows: Prepare the sample and place on the prism as previously described; read the instrument (% sugar); read the temperature correction thermometer and apply the correction to the figure just read; convert the corrected figure to per cent egg solids using the equations given in Table III. This instrument, combined with the electrolytic treatment of the egg, provides a rapid, convenient, and inexpensive method for determining the solids content of egg in industrial practice.

References

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis, 5th ed. A.O.A.C., Washington, D.C.　1940.
2. CAHN, F. J. and EPSTEIN, A. K.　U.S. Patent 2,065,114.　1936.
3. INTERNATIONAL COMMITTEE FOR UNIFORM METHODS OF SUGAR ANALYSIS.　Report of the Ninth Session.　1936.
4. URBAIN, W. M., WOOD, I. H., and SIMMONS, R. W.　Ind. Eng. Chem., Anal. Ed. 14 : 231-233.　1942.

PRECISION OF ASSESSMENT OF PALATABILITY OF FOODSTUFFS BY LABORATORY PANELS[1]

By J. W. Hopkins[2]

Abstract

Two categories of quality assessment of foodstuffs by panels of judges may be distinguished. In 'grading', an absolute assessment representative of the generality of consumers is sought. In 'analysis', maximum sensitivity is desirable and emphasis is shifted from absolute to relative assessments. In four series of 'grading' tests, individual ratings were most variable in the quality region close to the lower limit of acceptability, thus increasing to about 30 the calculable size of panel required to distinguish differences of the order of 5% from an assigned standard. The threshold concentration of primary taste substances detectable varies considerably between individuals, but except in extreme cases no consistent relation between taste acuity alone and palatability judgments was indicated. However, the judging characteristics of individuals may be investigated numerically by computing the correlation coefficients and regression equations relating their assessments to the average of those of all other members of the same panels. In this way a range of sensitivity of the order of 40% was demonstrable in the tests under review, making possible an objective evaluation of the suitability of individuals for 'grading' or 'analytical' tests.

Introduction

War conditions necessitated standardization and control of the quality of a number of bulk-produced foodstuffs, resulting, in certain instances, in increased requirements for assessment of their palatability in numerical terms. It is generally agreed that such assessments must be based in the last analysis on the reactions of human judges, for as has been pointed out by Platt (8), although the *quantity* of specific attributes of taste, odour, or texture may be capable of objective measurement, their *desirability* can only be determined subjectively. On the question of the precision of subjective assessments, however, opinion diverges widely. Thus whilst Crocker (4) states that "a considerable degree of reproducibility may be obtained in organoleptic testing" and that "even persons of ordinary discrimination may become good flavour discriminators if adequately trained", Crist and Seaton (3) conclude that "the ordinary tasting-panel method, as tested by the criterion of correlation in trials by duplication, is questionable. Either its improvement or its abandonment appears to be necessary and imperative".

Platt (8) draws a sharp distinction between assessments based on systems of scoring by a few specially trained or experienced individuals on the one hand, and direct tests of consumer preference on the other, the latter consisting of simple comparisons made by a large number of unselected people representative of the public providing the market for the product in question. Laboratory tests may themselves be subdivided in a similar manner, depending on the nature of the information sought. A commercial laboratory, for example, may wish to use a panel of tasters to grade the output of an already established product in order to prevent loss of goodwill through the sale of material deviating noticeably from the standard to which the public is accustomed. Such a panel need not comprise highly trained or expert personnel, but should consist of a small number of individuals who could be relied on to reproduce consistently the average reaction of the consuming public. What is desired here is in fact a reliable but rapid and inexpensive gauging of consumer tolerance, and excessive sensitivity would defeat this object. If on the other hand new or modified processes are being explored, laboratory palatability tests may be regarded as essentially analytical in nature. Greater sensitivity may therefore be desirable, and emphasis may be shifted from the absolute to the relative assessment of quality. Any such enhanced sensitivity must however still be in the right direction, i.e. a substance rated above-average by a panel of this type should also appeal to the general public. These considerations may be expressed in statistical terminology by stating that the taste reactions of individuals selected for laboratory panels for both grading and analytical work should be highly correlated with those of the generality of consumers. Furthermore, the average coefficient of regression of the scores assigned by individuals on the mean values resulting if all consumers made a similar test should be in the neighbourhood of unity for a grading panel. On the other hand, a high regression coefficient should be a condition of membership in an analytical panel. Clearly, the ratings of an analytical panel may always be used for grading purposes by the application of an appropriate conversion factor, but, in an institution undertaking any volume of organoleptic testing, it might be found desirable to reserve the more sensitive judges for discriminations of which the majority of the staff were incapable.

In practice, of course, perfect correlation is unattainable, but if the magnitude of the discrepancies between individuals is known, the size of panel required to produce an average assessment of specified accuracy may be calculated. A certain amount of information bearing upon this and the preceding points has accumulated from palatability tests made in these laboratories. This has accordingly been summarized and is reported below.

Analysis of Observations

Detection of Primary Tastes

Having regard to the demonstration by Blakeslee (1) and others of hereditary deficiencies in tasting ability, Knowles and Johnson (7) subjected 37 ran-

domly chosen prospective members of a palatability panel to a trial of their sensitivity to the four primary tastes, and reported the molar dilution in which each individual was able to identify the test substances used.   Two men and two women failed to detect the sweet taste in the highest test concentration of sucrose, one woman could not distinguish salt from sweet, and another was unable to identify bitter.   A parallel trial was subsequently undertaken in these laboratories in which similar test solutions were submitted to 56 individuals.   However, whereas Knowles and Johnson's subjects comprised 19 men and 18 women, in the later Ottawa test males predominated in the ratio of 42 men to 14 women.   Two of these 42 men could not detect bitter in the highest test concentration (0.05 M caffeine), three failed to detect the sourness of 0.003 M glutamic or tartaric acids, and one was unaffected by 0.05 M sucrose.   There was no case of complete 'taste blindness' among the 14 Ottawa women, but both sexes provided instances of incorrect identification of taste stimuli.   This was most pronounced in the case of tartaric acid (16 individuals), a circumstance in agreement with the observation of Knowles and Johnson (7) that "Bitter-sour indiscriminations in the low concentrations were frequent".

Both the Knowles and Johnson and Ottawa data were subjected to statistical analysis employing the probit-log dosage transformation found applicable to a wide range of physiological and psychological responses to a graded series of stimuli.   As was pointed out by Bliss (2), when the results for successive concentrations are merely different observations on a single set of individuals, the percentage reacting to a specified concentration can never be less than that recorded for a lower concentration, and successive observations are strongly correlated with each other; methods of computation appropriate to this situation were originally evolved by him for the analysis of time–mortality data in toxicological studies.   By this analysis it was found that in both groups of tasters the logarithms of individual threshold concentrations were approximately normally distributed.   Furthermore the median thresholds, at or below which 50% of individuals reacted, computed for the two groups separately and listed in Table I, agreed closely except in the case of caffeine (bitter), for which the observed difference exceeded three times its standard error.   This may reflect a real divergence in the sensitivity

TABLE I

COMPUTED MEDIAN THRESHOLD CONCENTRATIONS (MOLAR)

Trial	Sucrose (sweet)	Caffeine (bitter)	Glutamic acid (sour)	Tartaric acid (sour)	Sodium chloride (salty)
Knowles and Johnson (North Dakota)	0.0192	0.0008	0.0010	0.00026	0.0199
National Research Laboratories (Ottawa)	0.0195	0.0018	0.0008	0.00020	0.0192

of the tasters, but in view of the otherwise close agreement, the possibility of some lack of uniformity in the caffeine solutions used in the two tests cannot be excluded.

The variance of individual threshold concentrations in both groups and for all the test substances was of the same order, resulting in an average standard deviation of 0.3170 logarithmic units. In absolute units therefore, one would in general expect about 25% of all individuals to have thresholds exceeding the average in the ratio of 1.6 : 1 or more, whilst the median threshold of all persons below average in sensitivity would exceed the median of those of above-average sensitivity in the ratio of 3.3 : 1. Classification of the data from both laboratories in 2 × 2 contingency tables and calculation of the indices of association χ_c (6) listed in Table II revealed a significant degree of correlation in the sensitivity of the same individual to the sour, salt, and sweet

TABLE II

ASSOCIATION BETWEEN SENSITIVITIES TO DIFFERENT PRIMARY TASTES AS INDICATED BY χ_c FOR 2 × 2 CONTINGENCY TABLES

Test substance	Glutamic acid (sour)	Sodium chloride (salty)	Sucrose (sweet)
Caffeine (bitter)	2.16*	0.01	0.17
Glutamic acid (sour)		3.31**	3.23**
Sodium chloride (salty)			2.20*

* χ_c attains 5% level of statistical significance.
** χ_c attains 1% level of statistical significance.

test substances. Sensitivity to bitter was associated feebly with that to sour, and not at all with that to salt and sweet. In the same way, the one statistically significant value in Table III provided some suggestion that females detected glutamic acid more readily than did males, but provides no evidence of any effect of the smoking habit on taste acuity.

TABLE III

VALUES OF χ_c FOR 2 × 2 CONTINGENCY TABLE CLASSIFICATIONS OF INDIVIDUALS BY SEX AND ADDICTION TO TOBACCO, AND SENSITIVITY TO PRIMARY TASTES

Test substance	Males and females	Smokers and non-smokers
Caffeine (bitter)	0.03	1.27
Sucrose (sweet)	1.37	0.01
Glutamic acid (sour)	2.48*	0.01
Tartaric acid (sour)	1.39	0.00
Sodium chloride (salty)	1.69	0.01

* χ_c attains 5% level of statistical significance.

Knowles and Johnson (7) concluded that their results showed the necessity of testing the tasting ability of individuals before making selections for a panel of judges, and suggested criteria for the discrimination of "excellent", "good", and "fair" sensitivity, the last requiring the distinguishing of three of the four primary tastes in average or lower concentrations and eventual identification of the remaining taste. It must be agreed that it is difficult to imagine an individual notably deficient in sensitivity to two or more of the primary tastes functioning effectively as a judge of palatability. However, it must also be remembered that the sensation of palatability results from a combination of gustatory, olfactory, and tactile perceptions, the outcome of which is further conditioned by the subjective reaction of each individual to these various stimuli. Except in extreme cases, therefore, it would be too much to hope that this could be predicted from a single basic test.

Primary Tastes in Flavour Tests.

In order to provide some information respecting the extent to which sensitivity to the primary tastes affected judgments of flavour, a special series of samples consisting of scrambled egg containing various amounts of the foregoing test substances were made up and submitted to 30 of the 56 individuals taking part in the sensitivity determinations in the Ottawa laboratory. These special samples were tasted in sets of four, comprising an unadulterated fresh egg control and three preparations of fresh egg containing one of the test substances in an amount (calculated on a moisture basis) below, approximately equal to or definitely above the median threshold in the preceding trial. Such sets of four samples were included without identification in an extended series of flavour tests of scrambled egg made from dried egg powders, and were accordingly allotted scores by the tasters on the integral scale of 0 to 10, then used to assess the latter. In this way each of the 30 individuals participating tasted 20 special samples, of which five were controls and 15 were mixtures as described.

When analysed according to the procedure of Fisher (5), the total variance of the resulting 600 scores yielded the components shown in Table IV. These demonstrate that the average scores assigned by individual tasters to the entire series of samples varied appreciably, but that at the same time some statistically significant discrimination between the groups of samples containing different test substances, and between concentrations of these substances, was effected. The last component of variance shown in Table IV may be regarded as a measure of the basic error of tasting, namely inconsistency in the scoring of successive samples of the same material, and may be supposed to depend on acuity of perception and constancy of subjective reaction only. Relative scores allotted to samples actually differing in quality, on the other hand, involve judgment as well as perception, and consequently might be expected to exhibit more individual fluctuation. That this was not forthcoming in the present series of observations (Table IV) may have been because the differences in flavour between these test samples, although statistically demonstrable by reason of the large number of tasters

TABLE IV

ANALYSIS OF VARIANCE OF TASTERS' SCORES—FRESH EGG WITH AND WITHOUT ADDED SUBSTANCES

Source of variance	Degrees of freedom	Mean square
Between groups of samples	5	8.98**
Between samples of fresh egg	4	1.12
Between concentrations of added substances (average for all substances and tasters)	2	19.97**
Differential reaction to concentrations of individual substances (average for all tasters)	8	3.04
Between tasters (average for all samples)	29	26.56**
Differential reaction of individual tasters to groups of samples	145	1.69
Differential reaction of individual tasters to concentration of added substances	290	1.87
Differential reaction of individual tasters to samples of fresh egg	116	1.88

** *Exceeds mean square residual, 1% level of significance.*

employed, were not pronounced, and in fact corresponded to an average of less than 1 unit on the scale of assessment adopted (see Tables V and VI).

It is to be observed from Tables V and VI that, excepting one anomalous result with sucrose, the high concentration of the added substances alone was detected with any consistency, and resulted in an average reduction of palatability rating of 0.7 units. Divergence of the scores allotted to the same materials by different tasters (Table V) was however of an altogether greater order of magnitude, individuals' averages for the five control samples of fresh egg ranging from 10.0 to 5.4, and for all 20 test samples from 10.0 to 5.6. The individual (No. 10 in Table V, a male aged 39) who bestowed the maximum score on all samples indiscriminately was, as might be expected, relatively insensitive to all the primary tastes, his recorded thresholds for the test solutions exceeding the medians listed in Table I in the ratio of 4.4 for bitter, 1.3 for sweet, 2.5 for sour, and 1.6 for salty. However, taster No. 8 (a 26-year-old female) who reacted most unfavourably to the test samples, although of more than average sensitivity to sour and sweet, had an above-median threshold for bitter and was unable to identify the salty solution in the preceding trial; and, in general, correlation coefficients ranging from +0.02 (sweet) to +0.28 (bitter) were indicative of no significant association between an individual's logarithmic threshold as given by the primary test solutions and the scores subsequently allotted by him to the groups of egg samples containing the same taste substances. This finding is in agreement with the consideration, already noted, that gustatory perception is only one of several factors influencing palatability judgments.

Discrepancies between individuals' scoring of the same samples affects the reliability of palatability tests of the grading type, for in these an absolute score is required, and the panel of judges must accordingly be regarded as a theoretically random sample of the general consuming public. In self-

TABLE V

AVERAGE SCORES ALLOTTED TO GROUPS OF SAMPLES BY INDIVIDUAL TASTERS

Taster No.	Fresh egg (5 samples)	Fresh egg with addition of:					Average (20 samples)
		Sucrose (3 samples)	Caffeine (3 samples)	Glutamic acid (3 samples)	Tartaric acid (3 samples)	Sodium chloride (3 samples)	
1	7.6	8.0	7.3	8.3	7.7	7.7	7.7
2	8.0	2.3	8.0	6.7	8.0	8.3	7.0
3	8.2	8.3	8.0	7.7	7.3	8.3	8.0
4	8.8	8.7	5.3	8.0	6.7	8.3	7.5
5	9.4	8.3	10.0	9.3	9.3	10.0	9.4
6	8.8	8.3	7.7	7.7	8.0	8.7	8.3
7	10.0	8.7	10.0	10.0	10.0	9.3	9.7
8	5.6	4.7	5.7	6.7	5.0	5.7	5.6
9	8.0	7.7	8.7	7.7	7.7	7.7	7.9
10	10.0	10.0	10.0	10.0	10.0	10.0	10.0
11	7.0	6.7	7.3	6.0	7.7	7.0	7.0
12	8.2	7.7	8.3	8.7	9.0	8.3	8.4
13	7.2	4.3	4.0	6.3	7.3	7.7	6.3
14	7.6	6.0	6.7	8.0	8.0	6.0	7.1
15	10.0	8.0	10.0	9.3	8.3	10.0	9.4
16	8.0	6.0	5.0	7.3	7.7	8.3	7.2
17	7.8	8.0	7.3	7.7	7.3	7.3	7.6
18	5.4	6.3	5.7	7.3	5.3	8.3	6.1
19	8.4	6.3	8.7	6.0	7.3	8.0	7.6
20	9.4	10.0	10.0	9.7	8.7	9.7	9.6
21	6.0	7.0	5.7	6.3	5.0	6.0	6.0
22	10.0	8.7	8.7	9.3	9.7	9.3	9.4
23	8.8	7.7	8.3	8.3	8.3	9.0	8.5
24	6.8	5.0	5.3	8.3	6.3	8.7	6.8
25	9.4	8.0	7.3	7.7	8.6	7.3	8.2
26	8.8	8.3	8.3	8.7	7.3	9.3	8.5
27	8.0	7.7	8.0	7.7	8.0	7.0	7.7
28	7.0	8.0	6.7	7.3	8.0	7.3	7.4
29	6.2	7.7	6.7	7.3	6.7	7.7	7.0
30	7.4	8.3	6.3	7.7	9.0	8.0	7.7
Average	8.1	7.4	7.5	7.9	7.8	8.1	7.8

NOTE: *Necessary difference between averages of fresh egg and other groups* = 0.36.
Necessary difference between averages of other groups = 0.40.
Necessary difference between averages of individual tasters = 0.9.

contained analytical tests on the other hand, if an entire series of samples is assessed by the same judges, average differences between the ratings given by individuals to the series as a whole do not enter into comparisons of the relative scores given to specific samples. These are affected only by errors arising from sources of the type itemized in the last three lines of Table IV.

Variance of Palatability Assessments in Relation to Quality Level

It is of evident practical importance to know whether the variance of individual judgments of palatability, as expressed in numerical scores, is uniform over the range of quality encountered, and also whether some materials are productive of greater disagreement between individuals than others. Table VII summarizes the experience of this laboratory relevant to these points

TABLE VI

EFFECT OF CONCENTRATION OF ADDED SUBSTANCES ON SCORE (AVERAGE OF 30 TASTERS)

Substance	Concentration			
	Zero	Low	Medium	High
Sucrose	8.1	6.9	7.8	7.4
Caffeine	8.1	7.7	7.8	7.0
Glutamic acid	8.1	7.9	8.2	7.6
Sodium chloride	8.1	8.4	8.5	7.3
Tartaric acid	8.1	7.8	8.1	7.5
Average	8.1	7.7	8.1	7.4

NOTE: *Necessary difference between averages* = 0.3.
Necessary difference between individual items and zero = 0.5.
Necessary difference between individual items other than zero = 0.7.

TABLE VII

STANDARD DEVIATION OF INDIVIDUAL ASSESSMENTS AT VARIOUS QUALITY LEVELS

Test substance	Statistic	Average score									
		10.0–9.1	9.0–8.1	8.0–7.1	7.0–6.1	6.0–5.1	5.0–4.1	4.0–3.1	3.0–2.1	2.0–1.1	1.0–0.0
Butter (Panel of 17)	Standard deviation	—	0.84	1.15	1.23	1.30	1.49	1.62	1.63	1.11	0.84
	No. of samples	—	1	45	65	52	57	30	15	11	3
Dried eggs (Panel of 6)	Standard deviation	0.59	0.83	1.14	1.38	1.66	1.66	1.54	2.17	1.97	—
	No. of samples	2	41	108	139	64	59	19	10	2	—
Dried milk (Panel of 14)	Standard deviation	—	—	0.97	1.12	1.22	1.35	1.50	1.49	—	0.59
	No. of samples	—	—	3	60	90	70	27	12	—	2
Ration biscuits (Panel of 16)	Standard deviation	—	1.10	1.25	1.36	1.61	1.76	1.84	1.56	1.98	—
	No. of samples	—	28	190	206	160	73	17	2	1	—

resulting from a fairly extensive series of palatability tests of four foodstuffs, namely ration biscuits, dried eggs, butter, and dried milk. The standard deviations shown in this table are appropriate to absolute scores, i.e., to the grading type of test, and thus include any average differences between tasters.

On the whole, the degree of variability indicated was of the same order for all four substances. Furthermore, in all four cases individual scores became clearly less consistent as quality decreased, and were possibly most discrepant in the region corresponding to scores of 2 to 5. Owing to the infrequency of samples of the lowest quality, the zone of maximum uncertainty is not well determined. The existence of such a zone in any extended series including extremely good and extremely bad samples is however implicit in the bounding of the assignable scores.

It should be noted that an increase in individual variability necessitates the employment of more judges to produce an average assessment of specified reliability. In order to equalize the variance of the scores for dried egg given in Table VII for example, nearly eight times as many judges would be required in the quality range 2 to 3 as in 8 to 9. Deductions may also be made from Table VII respecting the actual number of judges required to yield average scores of specified accuracy. Thus in order to detect, at the 5% level of statistical significance (5), samples of dried egg differing from a pre-assigned quality rating of 4.0 by 1 unit or more on the quality scale here employed, 12 judges would be needed; whilst the detection of deviations of 0.5 unit would require the averaging of 46 individual scores. Discrimination between two samples of this mediocre quality rated by different panels would call for 23 judges per sample for a necessary difference of 1 unit and for 92 per sample for a necessary difference of 0.5 unit.

Correlation of Individuals' Assessments with Panel Averages

In an introductory paragraph it was pointed out that the suitability of individuals for laboratory taste panels might be evaluated statistically in terms of regression and correlation coefficients. Ideally, such evaluations should be made by relating each individual in question to the average of the consuming public. In practice, a reasonably representative sample of the latter must suffice. Even this was not available in the present instance, but, as the panels employed in this laboratory on the tests summarized in Table VII comprised scientific workers, technicians, and members of the administrative staff unselected in respect of tasting experience, training, or ability, correlation and regression coefficients of several individuals relative to all other panel members assessing the same samples have been computed as a matter of interest, and are shown in Table VIII.

Generally speaking the highest correlation coefficients resulted from the tests of ration biscuits, and the lowest from those of dried milk. However, in fairness to the tasters employed on the latter it should be noted that the great majority of samples of these fell within a narrow range of quality (see Table VII), so that discrepancies between individuals would be expected to constitute aft increased proportion of the total variance. An appreciable range is to be observed in individuals' correlation coefficients for butter (0.66 to 0.88) and dried milk (0.44 to 0.68), whilst ration biscuits gave slightly more uniform results (0.74 to 0.84). A somewhat higher degree of correlation is obviously desirable, and would operate to reduce the rather large numbers of tasters specified in the preceding section for results of statistical significance. Nevertheless this aspect of the tests was by no means wholly unsatisfactory, and certainly lends no support to the pessimistic views of Crist and Seaton (3) respecting the non-reproducibility of test panel results.

The regression coefficients listed in Table VIII, which as noted above provide a measure of the sensitivity of individuals' reactions, vary from 0.72 to 1.11 for butter, from 0.69 to 1.10 for dried milk, and from 0.68 to 1.14 for ration biscuits. For all three substances therefore, the range was of the order

TABLE VIII

CORRELATION OF INDIVIDUALS' ASSESSMENTS WITH AVERAGES OF REMAINDER OF PANEL

Test substance	Taster	No. of samples	Correlation coefficient	Regression coefficient	Average deviation of assessments from all others
Butter	A	130	.78	0.93	−0.26
(Panel of 17)	B	142	.88	1.09	−0.50
	C	140	.87	1.16	+0.06
	D	136	.76	0.89	−0.04
	E	127	.66	0.72	−0.54
	F	116	.81	1.11	+0.56
	G	128	.77	0.80	−0.15
	H	147	.76	0.94	−0.87
	I	117	.77	1.02	+0.06
Dried milk	A	307	.64	1.05	+0.52
(Panel of 14)	B	248	.54	0.95	−1.12
	J	250	.67	0.91	−0.42
	K	263	.68	1.10	−0.17
	L	296	.44	0.80	−0.45
	M	226	.54	0.86	+0.24
	N	256	.66	0.90	+0.46
	O	189	.47	0.69	+0.97
	P	222	.67	1.03	+0.46
Ration biscuits	A	157	.83	0.91	+0.70
(Panel of 16)	B	157	.78	1.04	−0.55
	C	139	.83	1.14	+0.09
	G	167	.75	0.68	+0.62
	J	166	.84	0.94	−0.12
	K	167	.80	0.85	−0.12
	Q	147	.74	0.96	+1.19

of 40% of the mean. The last column of Table VIII shows the amount by which the average score given by each judge exceeded or fell below the average of all other panel members assessing the same samples, and provides some further examples of consistent differences in individual preference levels of the type illustrated in Table V. The correlation coefficient, regression coefficient, and average score together provide a numerical characterization of the performance of the various judges. Taster C in Table VIII, for example, with a correlation coefficient of 0.87, a regression coefficient of 1.16, and an average preference level differing by only +0.06 from the remainder of the panel would be an obvious choice for the analytical assessment of butter, whilst E, with a correlation coefficient of 0.66, a regression coefficient of 0.72, and a mean deviation from the average preference of −0.54 scale units, equally obviously would not. Taster C also had the highest regression coefficient of those members of the ration biscuit panel investigated. Taster B had regression coefficients in excess of unity for both butter and ration biscuits but also exhibited a mean deviation from the average preference level of about −0.5 units in both instances, again illustrating the necessity of maintaining panel personnel unchanged throughout any series of samples for which maximum precision of relative assessment is desired.

Conclusions

The results described above may be summarized as follows:

There is appreciable variation in the ability to detect primary taste substances, the logarithms of individual threshold concentrations being approximately normally distributed. However, assessments of the palatability of foodstuffs depend upon olfactory and tactile as well as upon gustatory sensations, and are further conditioned by the subjective reaction of individuals to these stimuli. Except in extreme cases therefore, no consistent relation between taste acuity alone and palatability judgments is to be anticipated.

Two categories of quality assessment of foodstuffs by panels of judges may be distinguished. In the 'grading' type of test an absolute assessment is sought, and the panel must provide a representative sample of the reaction of the generality of consumers to the product in question. In tests of the 'analytical' type the effects of new or modified processes are compared, maximum sensitivity is desirable, and emphasis is shifted from absolute to relative assessments.

Analogous considerations apply to the fluctuations affecting the reproducibility of assessments, and a distinction may be made between consistent differences in preference level on the one hand, and the differential reaction of individual judges to particular samples on the other. Providing an entire series of samples is assessed by the same judges, the former may be eliminated from comparisons made within a test of the 'analytical' type. They cannot however be excluded from assessments on an absolute scale, in which they must be expected to constitute a major source of error.

In a series of tests in this laboratory in which quality was assessed on a numerical scale of 0 to 10, individual ratings became progressively more erratic as quality deteriorated, and were judged to reach a maximum of variability in the quality region corresponding to average scores of 2 to 5. This circumstance has the effect of increasing considerably the size of panel required to produce results of statistical significance when grading products of a quality close to the lower limit of acceptability.

The assessment characteristics of individuals may be investigated numerically by calculation of the coefficients of correlation and regression relating their scores to the average of those of all other members of the panel, and also by determining the mean amount by which their preferences are in excess or defect of the remaining panel average. In the tests considered here there was appreciable variation in both the correlation and regression coefficients of individuals, the latter indicating a range of sensitivity of the order of 40% of the mean. By statistics such as these the suitability of individuals for grading or analytical panels may be objectively evaluated, although in the case of grading panels it must always be borne in mind that the aim is to produce assessments characteristic of the consuming public in general rather than of the laboratory staff in particular.

Acknowledgments

Mr. C. O. Fulton and Drs. N. E. Gibbons and J. A. Pearce of these laboratories were responsible for the experimental work reported upon. Mr. D. B. W. Reid assisted in the compilation and analysis of the results.

References

1. BLAKESLEE, A. F. Proc. Natl. Acad. Sci. U.S. 18 : 120-130. 1932.
2. BLISS, C. I. Ann. Applied Biol. 24 : 815-852. 1937.
3. CRIST, J. W. and SEATON, H. L. Food Research, 6 : 529-536. 1941.
4. CROCKER, E. C. Flavor. McGraw-Hill Book Co., Inc., New York. 1945.
5. FISHER, R. A. Statistical methods for research workers. 8th ed. Oliver and Boyd, London. 1941.
6. FISHER, R. A. and YATES, F. Statistical tables for biological, agricultural and medical research. Oliver and Boyd, London. 1938.
7. KNOWLES, D. and JOHNSON, P. E. Food Research, 6 : 207-216. 1941.
8. PLATT, W. Food Research, 2 : 237-249. 1937.

ASCORBIC ACID CONTENT OF TOMATO VARIETIES AND ITS RETENTION IN PROCESSED PRODUCTS[1]

C. C. STRACHAN[2] AND F. E. ATKINSON[3]

Fruit and Vegetable Products Laboratory, Dominion Experimental Station, Summerland, B.C.

Tomatoes are one of the commonly available but variable sources of ascorbic acid. Canned tomatoes and tomato juice are the most important natural sources of ascorbic acid being manufactured in volume in Canada at a reasonable cost to consumers. If properly grown and prepared, these products should be not only attractive and palatable but also a good source of ascorbic acid. Reports published in April, 1944, and in December, 1944, by the Combined Food Board on food consumption levels in the United States, Canada and the United Kingdom showed that in Canada the only vitamin supply seriously deficient was ascorbic acid.

The outstanding value of the tomato in nutrition is due in great measure to its ascorbic acid (vitamin C) content. Published analyses indicate the extreme range that may be found in ascorbic acid content of tomatoes from various sources and at different times of the year. This range is from 5 to 50 milligrams per 100 grams of fresh material. However, the normal range of commercially grown summer varieties is probably 15 to 30 milligrams.

REVIEW OF LITERATURE

There are several published accounts on the ascorbic acid content of varieties and strains of tomatoes, particularly as they occur in the United States. Also a number of investigators have reported on several factors which may be responsible for fluctuations in ascorbic acid content. Excellent reviews on the subject are given by Hamner and Maynard (9), and Maynard and Beeson (17), and corroborated by more recent works. It is apparent that the principal factors affecting ascorbic acid value of tomatoes are variety (genetic factors) and climate, especially light. The latter appears to be of greatest influence the last two weeks of ripening time. Excessive heat may possibly have an adverse effect on the ascorbic acid content according to Reid (20).

A number of papers on the effect of processing on the vitamin C content of tomato juice appeared from 1930 to 1935, employing the bioassay method. These reports, notably Kohman, Eddy and Gurin (13) showed experimentally that if tomato juice were aerated during extraction much of the vitamin C was lost. However, if the tomatoes were crushed and boiled before extraction, or if the juice were immediately subjected to a vacuum, losses were small. Daggs and Eaton (7) examined the manufacture of one brand of commercially canned tomato juice reporting that tomato juice may be canned with little or no loss. By the methods employed at that time, it was difficult to detect small losses even if present.

Recent papers by Tressler and Curran (22), Maclinn and Fellers (16), Fellers and Buck (8), and Hauck (10), employing chemical methods for estimating ascorbic acid, have dealt with certain factors affecting retention of ascorbic acid in preparation and storage of home-processed and experimentally packed tomato juice. Lueck and Pilcher (15) presented information on various factors affecting retention of vitamins and flavour in canned fruit juices. A search of the literature has failed to reveal any comprehensive studies on the effect of processing methods under factory conditions on the retention of ascorbic acid in commercially canned tomato juice or tomatoes.*

A survey in 1942 by the Council on Foods of the American Medical Association (1) of a number of brands of tomato juice canned in the United States, showed a range of from 10 to 28 mg. per 100 grams of juice. The Eastern District (Philadelphia to Buffalo) was low with 10.4 to 13.3 mg., the Central District (Cincinnati to New Orleans) showed 14.9 to 20.7 mg. and the Western District (San Francisco-Denver-Seattle) gave values of 20.7 to 28.0 mg. In 1943 the East and Central Districts showed approximately the same ascorbic acid values and the West was again markedly higher. Another report by the Council on Foods (2) stated that the loss in canning is appreciable in tomato juice and intimated that improvement in the processing of this juice could be anticipated.

MATERIALS AND METHODS

In 1941 studies were commenced by this laboratory on the influence of processing factors on the retention of ascorbic acid in canned tomato juice. Advantage has also been taken of tomato varietal studies to determine the influence of variety on the ascorbic acid content of the canned product. In view of the definite need for maximum retention of vitamin C in commercial canned tomato juice, studies of the various steps in processing

FIGURE 1. Tomato variety test plots at the Dominion Experimental Station, Summerland, B.C.

* See footnote on page 94.

were made under actual operating conditions at several factories in 1943 and 1944. Furthermore, an extensive survey of the ascorbic acid content of canned tomato juice and tomatoes from the three commercial producing areas in Canada, namely, Quebec, Ontario and British Columbia, was made in 1944. This paper presents results of these studies to date.

The tomatoes employed in the study of varietal differences were grown side by side on experimental plots at the Summerland Experimental Station under good cultural and fertilizer conditions on light loam soil. In a number of cases, varieties were grown in the same area over a period of 3 to 4 years. They were harvested at correct canning maturity, prepared and canned in 28-ounce cans in the normal manner, care being taken to exhaust to 160° F. at the centre of the packed can prior to sealing. Two to 3 cans were used for analysis in each case unless otherwise noted. Employing the canned product for determination of the ascorbic acid content of the variety has several advantages, notably from the point of view of convenience and reduction of sampling error. The loss of ascorbic acid on canning under good conditions is insignificant and is comparable for all varieties being tested. Replicate cans were also examined to determine the canning characteristics of each variety. Results of these tests will be reported later.

In testing the influence of processing methods on the ascorbic acid content of tomato juice, 7 or more pounds of tomatoes, usually Earliana 8040, were used to prepare each lot of juice. The basic method of preparation was that described by Atkinson and Strachan (3) for home canned tomato juice. A stainless steel steam jacketed kettle was used for heating the tomatoes and juice to desired temperatures.

In 1944, in co-operation with the Canning Division of the Marketing Service, Dominion Department of Agriculture, 318 samples of tomato juice and 88 samples of canned tomatoes were obtained from the three commercial producing areas in Canada (Quebec, Ontario and British Columbia). These samples were analysed for ascorbic acid and proximate chemical composition and were examined for flavour, colour and other characteristics. Notes were also made of net weight of contents, head space, and vacuum. Only ascorbic acid determinations are being reported in this paper.

Ascorbic acid (reduced) was determined by the sodium 2,6-dichlorophenol indophenol dye visual titration method of Bessey and King (4), employing a 5- to 7-second end point. Daylite fluorescent light and white base were used to increase the accuracy of the end point determination. The dye was standardized according to the method of Buck and Ritchie (5). Occasionally standardization was carried out against pure crystalline ascorbic acid, good agreement being obtained by both methods. The extractions were made with 2% metaphosphoric acid or 0.4% oxalic acid (Ponting, 18).

For juice, 50 ml. were pipetted into a 250-ml. volumetric flask containing extractant, made up to volume with acid extractant, shaken thoroughly and filtered. Five to 15 ml. aliquots were then titrated rapidly with the dye and results reported as mg. per 100 ml. of juice. Where necessary, for certain comparative purposes, the determinations were recalculated to weight basis from the specific gravity of the juice. In nearly all tests, samples were taken in duplicate or triplicate.

For canned tomatoes, determinations were carried out as for juice on 50-gram portions after mixing of the whole contents of the can in a Waring blendor for 30 seconds.   This operation permitted more accurate sampling. Tests showed no ascorbic acid was lost by this procedure.   In fact, blending 3 to 5 minutes resulted in no decrease of ascorbic acid.   Unless otherwise noted 2 or 3 canned samples were examined from each lot or test.

Fresh fruit was prepared for analysis by cutting quarters from 4 fruits to make 100 grams and extracted with 400 ml. of oxalic or meta-phosphoric acid extractant in a Waring blendor for 2 minutes and filtered through No. 4 or No. 12 Whatman filter paper.   Five to 15 ml. aliquots of the filtrate were titrated with the dye.   This method is essentially that described by Loeffler and Ponting (14).   The procedure was repeated three or more times for any sample of fruit so that at least 12 representative fruits and usually more were used for each sample, the mean of the individual determinations being recorded.

Tests for interference of metallic ions in the canned product to the dye titration showed that there was none.

RESULTS
The Effect of Variety and Season
In Tables 1 and 2 are presented the ascorbic acid values for 31 different varieties and strains of tomatoes grown under the same conditions at the Summerland Experimental Station.   It will be noted from these tables that there was marked variation in ascorbic acid content of varieties.   Also while there was considerable variation in ascorbic acid values in the same variety from year to year and even from one picking to another, the varieties tended to maintain their relative position of one to another especially where average difference was marked.   These results are in general agreement with the current literature on this question.   The Signet variety developed at the Summerland Station is outstanding in its consistently high ascorbic acid content having a 3-year average of 29.8 mg. of ascorbic acid.   Its only serious fault is that it lacks size for a good commercial canning tomato.   Clarks Early and Sugawara have proved to have consistently good ascorbic acid values but are not outstanding.   Fortunately, Clarks Early and Sugawara have good cultural and canning characteristics which make them satisfactory canning varieties under actual commercial conditions in the Okanagan Valley and adjacent areas.   Harkness and Bestal (Sd.) have shown consistently fairly good ascorbic acid values but are not satisfactory for other reasons.   Master Marglobe, Marglobe × Bonny Best, Stokesdale, Valiant, Livingstone Globe × Gnome and Essary, while having on limited trial apparently good ascorbic acid content, are unsatisfactory in other respects, either not suited to this growing area or else unsatisfactory for canning.   California Dawn, a good sized tomato and a satisfactory canner, had on 2 years tests a fairly good ascorbic acid content.

In general, the ascorbic acid contents of the varieties recorded in Tables 1 and 2 compare favourably with the higher results for the same varieties reported in the literature.   These high values are probably due in part to favourable climate with respect to temperature and light as

indicated in studies by Reid (19, 20, 21), Wokes and Organ (23) and Kaski, Webster and Kirch (11).   It is interesting to note from Table 1 that under conditions prevailing, there was no consistent difference in the ascorbic acid content of tomatoes harvested early and late in the season.

TABLE 1.—ASCORBIC ACID CONTENT OF VARIETIES AND STRAINS OF TOMATOES
HARVESTED AT SEVERAL DATES OVER A PERIOD OF THREE TO FOUR YEARS

Variety or strain	Ascorbic acid content in milligrams per 100 grams							Average for 3 or 4 years
	Dates tomatoes harvested and canned							
	1941	1942	1943			1944		
	Sept. 4	Sept. 22	Sept. 10	Sept. 29	Oct. 19	Sept. 6	Sept. 27	
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Signet	33.1	—	23.7	29.1	34.9*	24.4	33.7	29.8
Clarks Early	—	31.7	20.9	24.1	23.2*	—	18.9	23.8'
Harkness Early	24.7	19.7	24.2	25.1	—	—	—	23.4
Sugawara	24.5	26.3	22.8	25.7	22.0*	20.4	19.0*	23.0
Bestal (Sd.)	23.3	24.7	20.8	21.2	—	—	—	22.5
Dick Locke (Round)	—	22.1	17.4	23.0	23.4	20.5	20.0†	21.1
Signet × Clarks Early (Sd.)	16.4	24.6	20.1	21.5	—	—	—	20.7
Sentinel (Sd.)	—	24.1	16.0	21.7	16.6*	21.4	22.8	20.4
Bounty	16.6	20.7	—	—	—	15.8*	20.8	18.5

* One sample only.
† Another sample from quite different soil (Trout Creek) contained 22.8 mg. ascorbic acid.

TABLE 2.—ASCORBIC ACID CONTENT OF TWENTY-TWO VARIETIES
AND STRAINS OF TOMATOES

Variety or strain	Ascorbic acid content in milligrams per 100 grams of canned product			
	1941	1942	1943	1944
	mg.	mg.	mg.	mg.
Master Marglobe (Stokes)	30.5	—	—	—
Marglobe × Bonny Best	30.2	—	—	—
Signet × Sugawara	—	—	—	30.0
Stokesdale (Stokes)	29.3	—	—	—
Valiant (Stokes)	28.3	—	—	—
Sugawara × Signet	—	—	28.1	27.5*
Asgrow Scarlet Dawn	—	—	—	23.8*
Globonnie	26.3	—	—	—
Livingstone Globe × Gnome × L. G. × Abel	26.2	—	—	—
Essary (Univ. of Tenn.)	25.5	—	—	—
Hybrid 46 (Mich.)	24.4	—	—	—
California Dawn	—	23.6	22.6†	—
8040 (Earliana Sport)	—	25.1	21.2*	—
Signet × John Baer	—	—	—	21.8*
Rutgers (Vineland)	21.5	—	—	—
Rutgers (Stokes)	21.5	—	—	—
Signet × California Dawn	—	—	—	21.7*
Abel	19.9	—	—	—
Bulman's Special (Flat)	—	18.8	20.9†	—
N.D.A.C.	17.9	—	—	—
Bison	17.3	—	—	—
Bulman's Flat (Sd. 993)	—	—	23.0*	15.2

* Average of two pickings.
† Average of three pickings.

The production of a high vitamin C, good quality, heavy yielding tomato with satisfactory canning and juice characteristics appears to be a very urgent problem for the plant breeder.   In this connection it is interesting to note that crosses made at the Summerland Station, using the high vitamin C variety Signet as one parent, have given encouraging results. Data presented in Table 3 indicate the possibilities of developing a variety combining high ascorbic acid content with superior cultural and canning characteristics. These data were obtained by analysing fruit from individual plants selected from among many in breeding plots at the Experimental Station.   These plants were originally selected on the basis of plant and fruit characteristics suitable for commercial market or cannery production.

TABLE 3.—ASCORBIC ACID CONTENT OF TOMATOES FROM
INDIVIDUAL PLANTS, 1943

Plant No.	Parentage	Ascorbic acid content mg. per 100 gm.
		mg.
1–43	John Baer × Signet	34.2
2–43	John Baer × Signet	23.9
3–43	Signet × Calif. Dawn	31.0
4–43	Signet × Calif. Dawn	20.8
5–43	Signet × Calif. Dawn	34.4
6–43	Sugawara × Signet	35.9
7–43	Sugawara × Signet	41.6
8–43	Signet × Sugawara	32.4
9–43	Signet × Sugawara	34.8

Ascorbic Acid Content of Commercially Canned Juice and Tomatoes

The results of a survey of the ascorbic acid values of tomato juice and canned tomatoes as commercially produced are presented in Table 4. There is considerable variation in the ascorbic acid content of tomato juice with somewhat less variation in values for canned tomatoes.   However, the most important points to note in this table are: (1) the significantly higher average ascorbic acid content of 22.3 and 19.8 mg. for tomato juice manufactured in British Columbia compared with 14.4 and 15.0 mg. for Eastern Canada; (2) the relatively high minimum values of 12.6 and 15.6 mg. for British Columbia produced tomato juice and the low minimum values of 8.7 and 4.3 mg. for Eastern packed juice while the maxima of both districts are more nearly comparable; (3) the minimum values for ascorbic acid in canned tomatoes are almost identical for both Eastern Canada and British Columbia.   These results suggest that many of the low values for tomato juice are to a large extent due to faulty processing and unsatisfactory equipment.   The average ascorbic acid values in canned tomatoes from widely separated districts suggest inherent differences which are probably due largely to climate.

TABLE 4.—ASCORBIC ACID CONTENT OF COMMERCIALLY CANNED TOMATOES
AND TOMATO JUICE IN CANADA

Year packed	Area	No. of samples analysed	Ascorbic acid values per 100 ml. of juice		
			Average	Maximum	Minimum
			mg.	mg.	mg.
JUICE					
1941	British Columbia	18	22.3	33.3	12.6
1942	Eastern Canada	12	14.4	19.5	8.7
1944	Quebec	44	14.2	26.0	6.1
	Ontario	220	15.1	25.0	4.3
	British Columbia	54	19.8	26.9	15.6
	Eastern Canada	264	15.0	26.0	4.3
TOMATOES			per 100 grams of tomatoes		
1940–41	British Columbia	39	21.6	32.4	14.4
1944	Quebec	5	15.9	18.9	14.1
	Ontario	30	17.2	20.3	15.1
	British Columbia	53	22.5	27.7	16.3
	Eastern Canada	35	17.0	20.3	14.1

*Comparison of Ascorbic Acid Content of Juice and Tomatoes Canned
Commercially*

In order to ascertain how the retention of ascorbic acid in tomato
juice compared with that of tomatoes, the data in Table 5 were compiled.
The factory from which the samples were secured was well equipped and
considered to be packing both products satisfactorily. Canned samples of
juice and tomatoes were taken on the same day and also at the same hour
when possible. The canned tomato samples obviously represent smaller
numbers of fruit than do the juice samples but it is believed there are
sufficient samples to make the results significant. Under good processing
conditions it would appear that on the average the tomato juice contains
about 14% less ascorbic acid than do comparable canned tomatoes. A
limited survey of samples from a number of other factories indicates that
losses much greater than this occur. Indications are that tomatoes lose
very little ascorbic acid in canning. Unpublished data of the Chemistry
Division, Science Service, Ottawa (6), showed no significant loss of ascorbic
acid in commercial canning of tomatoes.

*Studies on Retention of Ascorbic Acid in Tomato Juice Under Factory
Conditions*

In 1943 it was decided to study ascorbic acid retention under com-
mercial conditions by following the produce as received at the factory
through the various processing steps in the plant. This survey was carried
out in one plant that year with certain recommendations being made as to
improvements in the manufacturing line. The study was repeated in
1944 at the same factory as well as at two additional factories. The results

TABLE 5.—ASCORBIC ACID CONTENT OF CANNED TOMATOES AND TOMATO JUICE PACKED ON SAME DAY AT FACTORY C

(1944 season)

Sample number	Date packed	Ascorbic acid content per 100 grams	
		Juice	Tomatoes
		mg.	mg.
1	Sept. 14	16.7	18.5
2	Sept. 15	21.3	24.3
3	Sept. 19	20.3	22.9
4	Sept. 21	15.2	27.7
5	Sept. 22	21.5	18.9
6	Sept. 23	17.2	21.1
7	Sept. 25	22.5	23.2
8	Sept. 26	22.5	17.8
9	Sept. 27	18.6	· 21.7
10	Sept. 28	20.2	24.3
11	Sept. 30	19.1	21.1
12	Sept. 30	19.3	—
13	Oct. 2	21.4	23.2
14	Oct. 3	23.5	27.1
15	Oct. 5	23.2	23.4
16	Oct. 6	17.2	23.2
17	Oct. 7	21.2	22.2
18	Oct. 11	20.1	22.2
19	Oct. 13	18.4	20.9
20	Oct. 16	18.1	19.8
21	Oct. 16	15.7	—
22	Oct. 17	26.1	31.6
23	Oct. 18	17.0	24.4
Average		19.8	22.8
Maximum		26.1	27.7
Minimum		15.2	17.8

of the study at Factory C in 1944 are given in Table 6. This factory has what is considered to be a very good tomato juice manufacturing line. Losses were in general smaller and less variable at this plant than in the other two plants investigated. The ascorbic acid figures recorded in Table 6 for raw fruit are very approximate due to the fact that only 12 to 16 fruits were analysed whereas the other figures represent a volume of 50 to 100 gallons of juice or equivalent to around 700 to 1400 pounds of tomatoes. It is unlikely that there was a significant loss at this plant in the hot break due to the few seconds only required following milling to reach the inactivating temperature of 190° F. Hence this figure probably should be taken as representing more nearly the true average ascorbic acid value of the raw product being employed during the tests. On this basis the total loss in processing under good conditions was 2 to 3 mg. or 11.7 to 13.5%. Loss at Factory A was only very slightly greater than at C, but Factory B showed a loss of 18.1 to 26.9%. This was probably due to low temperature extraction allowing enzyme action together with aeration of the product. Losses greater than those found could be expected under such conditions. None of the factories studied permitted contact of the product with copper equipment.

TABLE 6.—RETENTION OF ASCORBIC ACID AT PROGRESSIVE STEPS IN
COMMERCIAL PROCESSING OF TOMATO JUICE

Test No.	Steps in processing	Ascorbic acid per 100 gm.
	FACTORY C (1944)	mg.
A 1	Raw tomatoes	25.0
2	Emerging from hot break at 200° F.	22.2
3	In finisher receiving tank at 188-190° F.	20.7
4	Holding-salting tank (100 gal.) at 183° F.	19.4
5	After filler prior to sealing can at 182° F.	19.3
6	After canned juice stored three weeks	19.2
B 1	Raw tomatoes	20.8
2	Emerging from hot break at 200° F.	19.6
3	In finisher receiving tank at 188-190° F.	19.2
4	Holding-salting tank (100 gal.) at 183° F.	17.2
5	After filler prior to sealing can at 182ª F.	16.3*
6	After canned juice stored three weeks	17.3

* Froth at time of sampling, indicating air in product, likely accounts for this low figure. More frothing seemed to occur with the less mature fruit.

Effect of the Method of Extraction on the Ascorbic Acid Content

Laboratory experiments were carried out to determine the effect of different temperatures employed in preheating the tomatoes prior to extraction and also the actual method of extraction on the ascorbic acid content of the resultant canned juice. The results obtained are reported in Table 7. All tests were made from the same lot of raw tomatoes; 7 to 10 pounds of tomatoes were used for each test. In test No. A, B, J, and K, the tomatoes were placed in a small stainless steel jacketed kettle, pulped while heating in about 5 minutes to 210° F. (boiling at this altitude) using 20-25 lb. steam pressure, boiled specified time, then extracted by various methods. To pass the cooked tomato pulp through the suspended screen by hand required about 3 minutes. Tests C and D were pulped and heated as for A but to lower temperatures. In test E, whole tomatoes were placed in boiling water, and in test F, whole tomatoes were exposed to flowing steam. In tests G, H, and I, whole fruit was extracted without any heating. In all cases, the juice obtained by the various methods of extraction was heated quickly in the kettle to 190° F. and the cans filled full at that temperature, sealed, processed in boiling water 10 minutes and then water cooled.

The very important point brought out in Table 7 is the necessity of rapid heating of the milled pulp to sufficiently high temperature (at least 190° F.) to inactivate quickly the relatively strong oxidase enzymes present in the tomatoes. If this is done, loss of ascorbic acid is relatively small but if not, the loss may be serious as indicated in Table 7 where as much as 36% loss occurred. The actual method of extraction appeared to be of little consequence provided the extraction was on pulp heated to 190° to 210° F. This temperature has the additional advantage that it also inactivates pectin-destroying enzymes resulting in improved consistency of juice as pointed out by Kertesz and Loconti (12).

TABLE 7.—EFFECT OF THE METHOD OF EXTRACTION ON THE ASCORBIC ACID CONTENT
OF EXPERIMENTALLY PREPARED CANNED JUICE

Test No.	Method of extraction	Ascorbic acid per 100 ml.	Per cent loss compared to Lot K
		mg.	%
A	Pulped and boiled 3 to 4 min. Extracted hot through screen	30.2	8.5
B	Pulped and boiled ½ to 1 min. Extracted hot through screen	29.6	10.3
C	Pulped and heated to 173° F. Extracted hot through screen	22.4	32.1
D	Pulped and heated to 110° F. Extracted immediately	22.9	30.6
E	Scalded whole 3 min. in boiling water, pulped and extracted immediately through screen	24.5	25.8
F	Scalded whole 2 min. in flowing steam, pulped and extracted immediately through screen	22.9	30.6
G	Pulped and cold extracted through screen	22.5	31.8
H	Pulped and cold extracted through centrifugal juicer	21.8	33.9
I	Pulped and cold extracted through screw expeller press	21.0	36.4
J	Pulped and boiled ½ to 1 min. Extracted hot through small centrifugal juicer	25.2	23.6
K	Pulped and boiled ½ to 1 min. Extracted hot through small screw expeller press	33.0	0.0

Effect of Sterilizing Temperature and Period

To study the effect of sterilizing temperature and time on the ultimate ascorbic acid content of canned tomato juice, several lots of juice were prepared according to Atkinson and Strachan (3) home process procedure. The results of this experiment are recorded in Table 8.   The cans in each lot were filled full at 190° F., sealed, sterilized as recorded in the table, and water cooled.   Analyses were made after several months storage at 50° F. One-half the cans from each lot of prepared juice were held hot on their sides in the air for 5 minutes or processed in boiling water for 10 minutes for controls.   The different lots were not always necessarily prepared from the same fruit.   The results show that the sterilizing temperature had very little effect on the final ascorbic acid content of canned tomato juice.   This is in agreement with limited data obtained in 1943 under commercial conditions.   It was observed that excessive processing temperatures and long processing time had a deleterious effect upon colour and flavour.

SUMMARY

Analyses for ascorbic acid content have been made of 31 varieties and strains of tomatoes grown under identical conditions at the Summerland Experimental Station.   A number of the varieties were examined over a period of 3 to 4 years.   Clarks Early and Sugawara were found to have consistently good ascorbic acid values and are satisfactory canning tomatoes in this area.   The Signet variety proved to be consistently high in ascorbic acid, having a mean value of 29.8 mg. per 100 grams over a 3-year period. Its other characteristics are also good with the exception that it tends to be small. · The fruits from individual plants, resulting from crossing Signet with larger fruited varieties, were analysed for ascorbic acid.   The results indicate the practicability of developing varieties combining high ascorbic acid content with superior cultural and canning characteristics.

TABLE 8.—EFFECT OF STERILIZING TEMPERATURE AND PERIOD ON ASCORBIC ACID
CONTENT OF EXPERIMENTALLY CANNED TOMATO JUICE

| Lot No. | Processing data | | Ascorbic acid per 100 ml. |
	Sterilizing process	Period	
		min.	mg.
1 (a)	210° F. (Boiling water)	10	26.2
(b)	(Held on side in air)	5	26.5
2 (a)	240° F. (Retort)	10	26.6
(b)	210° F. (Boiling water)	10	26.3
3 (a)	240° F. (Retort)	20	27.9
(b)	210° F. (Boiling water)	10	29.3
4 (a)	250° F. (Retort)	5	24.9
(b)	210° F. (Boiling water)	10	25.9
5 (a)	250° F. (Retort)	15	26.8
(b)	210° F. (Boiling water)	10	28.0

A comprehensive survey was made of commercially canned juice and tomatoes in Canada. Marked differences were found in average ascorbic acid content of tomato juice produced in British Columbia and Eastern Canada. In 1941 and 1944 the British Columbia mean values were 22.3 and 19.8 mg., respectively, while the 1942 and 1944 values for Eastern Canada were 14.4 and 15.0 mg. Very low values were found in tomato juice packed in Eastern Canada, yet these low values were not found in the canned tomato samples.

An extensive study was made at three factories of the retention of ascorbic acid in tomato juice under factory conditions. Analyses were made at several steps in the process of manufacture. This study revealed that under good processing conditions the total loss in processing from the raw fruit to the final canned product should not exceed 2 to 3 mg. Loss at many factories apparently greatly exceeds this figure.

Laboratory experiments were conducted on the effect of the method of extraction and the effect of sterilizing temperatures and time on the retention of ascorbic acid in canned tomato juice. The great importance of rapidly heating the milled tomatoes to 190° F.–210° F. prior to extraction was demonstrated. The sterilizing temperature and length of cook had insignificant effect on the ultimate ascorbic acid content of the canned juice. Excessive sterilizing did, however, adversely affect the colour and flavour of the juice.

ACKNOWLEDGMENT

REFERENCES

1. AMERICAN MEDICAL ASSOCIATION COUNCIL ON FOODS AND NUTRITION. The Canner 100 : 2 : Serial 2602, p. 12 (Dec. 9). 1944.

2. AMERICAN MEDICAL ASSOCIATION COUNCIL ON FOODS AND NUTRITION. J. Amer. Med. Assoc. 125 : 429 (June 10). 1944.

3. ATKINSON, F. E., and C. C. STRACHAN. Home processing of fruits and vegetables. Dept. of Agric. Pub. 744, Farmers Bull. 114. 1943.

4. BESSEY, O. A., and C. G. KING. The distribution of vitamin C in plant and animal tissues and its determination. J. Biol. Chem. 103 : 687-698. 1933.

5. BUCK, R. E., and W. S. RITCHIE. A new method for the standardization of the dye used for the determination of cevitamic acid (vitamin C). Ind. Eng. Chem., Anal. Ed. 10 : 26. 1938.

6. CHEMISTRY DIVISION, SCIENCE SERVICE, OTTAWA. Personal communication (June, 1944).

7. DAGGS, R. G., and A. G. EATON. Vitamin C content of canned tomato juice. Ind. Eng. Chem. 26 : 292-295. 1934.

8. FELLERS, C. R., and R. E. BUCK. Retention of vitamins C and A in glass-packed foods. Food Res. 6 : 135-141. 1941.

9. HAMNER, K. C., and L. A. MAYNARD. Factors influencing the nutritive value of the tomato. U.S. Dept. Agric. Misc. Publ. No. 502. 1942.

10. HAUCK, H. M. Ascorbic acid in home-canned tomato juice. J. Home Econ. 35 : 295-300. 1943.

11. KASKI, I. J., G. L. WEBSTER, and E. R. KIRCH. Ascorbic acid content of tomatoes. Food Res. 9 : 386-391. 1944.

12. KERTESZ, Z. I., and J. D. LOCONTI. Factors determining the consistency of commercial canned tomato juice. New York State Exp. Sta. Tech. Bull. 272. 1944.

13. KOHMAN, E. F., W. H. EDDY, and C. Z. GURIN. Vitamins in canned foods. XIII. Canning tomato juice without vitamin C loss. Ind. Eng. Chem. 25 : 682-684. 1933.

14. LOEFFLER, H. J., and J. D. PONTING. Ascorbic acid—rapid determination in fresh, frozen or dehydrated fruits and vegetables. Ind. Eng. Chem., Anal. Ed. 14 : 846-849. 1942.

15. LUECK, R. H., and R. W. PILCHER. Canning fruit juices—technical aspects. Ind. Eng. Chem. 33 : 292-300. 1941.

16. MACLINN, W. A. and C. R. FELLERS. Ascorbic acid (vitamin C) in tomatoes and tomato products. Mass. Agr. Exp. Sta. Bull 354. 1938.

17. MAYNARD, L. A., and K. C. BEESON. Some causes of variation in the vitamin content of plants grown for food. Nutrit. Absts. & Rev. 13 : 155-164. 1943.

18. PONTING, J. D. Extraction of ascorbic acid from plant materials. Ind. Eng. Chem., Anal. Ed. 15 : 389-391. 1943.

19. REID, M. E. The effect of light on the accumulation of ascorbic acid in young cowpea plants. Am. J. Botany 25 : 701-710. 1938.

20. REID, M. E. Relation of temperature to the ascorbic acid content of cowpea plants. Bull. Torr. Bot. Club 68 : 519-530. 1941.

21. REID, M. E. Effect of variations in light intensity, length of photo-period, and availability of nitrogen upon accumulation of ascorbic acid in cowpea plants. Bull. Torr. Bot. Club, 69 : 204-220. 1942.

22. TRESSLER, D. K., and K. M. CURRAN. The cause of loss of vitamin C from bottled tomato juice. J. Home Econ. 30 : 487-488. 1938.

23. WOKES, G., and J. G. ORGAN. Oxidizing enzymes and vitamin C in tomatoes. Biochem. J. 37 : 259-265. 1943.

---

* Since the preparation of this paper, two studies have been reported on factors influencing ascorbic acid retention in commercially processed tomato juice: (1) Clifcorn, L. E., "Variables influencing vitamin content of processed foods," The Food Packer, pp. 46-48, August, 1945, and (2) A Memorandum by the Research Department of the American Can Company on "Tomato juice—factors influencing ascorbic acid retention," 1945.

DRIED WHOLE EGG POWDER

XX. THE EFFECT OF GRADE OF EGGS, LOCALITY AND MONTH OF PRODUCTION, AND CLIMATIC CONDITIONS ON THE SOLIDS CONTENT OF LIQUID EGG AND ON THE QUALITY OF THE POWDER PRODUCED[1]

By Jesse A. Pearce[2], Margaret Reid[2], Miss B. Metcalfe[3], and H. Tessier[4]

Abstract

Grade *A* large eggs had the lowest average solids content (25.6%) of the grades studied and Grade *C* eggs had the highest (26.6%). The solids content increased during the period from December 1944 to July 1945. The total increase during this interval was about 0.5%.

The average potassium chloride value of powder produced from Grades *B* and *C* eggs was higher than the value for Grade *A* medium eggs; Grade *A* medium and pullet eggs produced powder better in this quality attribute than Grade *A* large (differences of about 2%). The use of Grade *C* eggs resulted in a powder with an average fluorescence value about 2 units greater than for powder from any other grade of eggs. The month of egg production affected quality measures on the resulting powder. As the season progressed there was a decrease in the fluorescence value and pH and an increase in potassium chloride value and foaming volume value.

The changes noted could not be attributed to climatic conditions, but may be attributable to feeding practices and to increased age of the hen.

Introduction

The increased production of dried eggs in Canada has focused attention on possible differences between lots of eggs that may affect both the yield and quality of the resulting powder. Recurring statements from some producers of powder indicated that eggs from different parts of the country varied widely in solids content. One processor claimed that eggs from one area consistently produced 4% less powder than eggs from another area. Therefore, it seemed advisable to attempt a monthly assessment of the solids content of various grades of eggs from different parts of the country.

There is some evidence that eggs produced between November and March are of the highest quality (3, 4, 5, 7, 12). Although these reports did not deal with solids content specifically, they describe decreases in quality from March to November as assessed by the condition of the thick white, the percentage of thick white, yolk index (4, 5), and candling appearance (7). There was no seasonal change in yolk colour (4, 5) but this may be attributed to the use of standard mash for feeding the experimental birds. One Canadian report indicates that the albumin index of eggs produced in March is higher than that of eggs produced in June (3).

[2] *Biochemist, Food Investigations.*

[3] *Laboratory Assistant, Food Investigations.*

[4] *Laboratory Steward, Food Investigations.*

Therefore, simultaneously with an assessment of solids content of the liquid eggs, some of the material was dried and the yield determined. The properties of the powder were assessed, using the quality tests currently applied to dried egg powder (6) and a test of baking properties (9). The colour of the powder was measured on a colour comparator (13). The present paper describes differences in solids content of the liquid eggs, and in yield and quality of the dried product. The colour measurements will be discussed as part of a subsequent paper on the colour of dried egg powder (10).

Materials and Methods

The original experimental design required the shipment of eggs from Vancouver, Edmonton, Winnipeg, London, Belleville, and Three Rivers. While these did not represent all the areas from which eggs are purchased for drying, they did represent the areas subject to controversy and were believed to be a good cross-section of Canada's egg producing districts. Since surplus eggs become available in Canada in December and production falls off the following July when the hens begin to moult, eggs were taken from current receipts on the 15th of each month from December 1944 to July 1945. This would represent the eggs most likely to be dried. Five dozen of each of the following grades were to be shipped at each sampling time: *A* large, *A* medium, *A* pullet, *B* and *C*. Since Canadian eggs can be classed as *B* and *C* for dirty shells as well as for quality (2), the samples representing these grades were selected on the basis of quality only. All eggs were to be drawn from current receipts in the areas designated, and samples were taken and graded by inspectors of the Special Products Board of the Canadian Department of Agriculture.

Unfortunately, the original plan could not be adhered to entirely. Only four areas could supply eggs in December 1944, and it was impossible to obtain the required *B* and *C* eggs from the Vancouver area. From time to time, single items were missing in the various consignments. Nevertheless, it was believed that the survey was sufficiently complete to permit conclusions of value. Altogether, 224 five-dozen lots of eggs were used in the study.

Upon receipt in the laboratory, the eggs of each of the various grades were broken and mixed; samples were then drawn for determinations of solids content (1, p. 308) and the remainder was weighed before going to the laboratory spray drier (14) for dehydration (drying temperatures: inlet, 250° F.; outlet, 140° F.). After dehydration the material from the main chamber and the secondary collector were weighed separately, and the moisture contents determined (11). From these values, the recovered solids were calculated as a percentage of the solids introduced into the drier.

Only the powder from the main chamber was used for measurement of fluorescence value (8), potassium chloride value (11), pH (6), and foaming volume (9).

Results

The significance of the results was assessed by analyses of variance. Two comparisons were made: the first compared Grade A eggs of all sizes from the six areas for the months of January to July 1945; the second compared Grade A medium eggs with Grades B and C eggs for the same collection times but for five areas only. Since the mathematical treatment showed little evidence of differential behaviour, the presentation of the results has been simplified by the use of tables of means and necessary differences (Tables I, II, and III). The effect of area of production on solids content of Grade A eggs almost attained significance and was given further consideration (Table IV). There appeared to be some relation between quality and time of production and possibly some relation between quality and climatic conditions, shown by tables of correlation coefficients (Tables V and VI).

Solids Content and Yield

The results indicated that Grade A large eggs had a significantly lower solids content than Grade A medium and Grade A pullet eggs, while Grade C eggs had the greatest solids content (Table I). Area of production had a significant effect on the solids content of Grades B and C eggs and the effect almost attained significance for Grade A eggs (Table II). Although the differences are small, there is generally a tendency for solids content to increase as the season progresses (Table III). This would correspond to a previously observed seasonal trend in loss of quality before the candling lamp (7). One particular feature is increase in the size of the air cell that is believed to accompany loss of moisture through the shell.

TABLE I

RELATION BETWEEN GRADE OF EGGS, SOLIDS CONTENT, AND QUALITY OF EGG POWDER PRODUCED DURING A SEVEN MONTH PERIOD

Grade of eggs	Solids content, %	Quality of powder			
		Fluorescence value	Potassium chloride value, %	pH	Foaming volume, ml.
Averages for eggs produced in six areas					
A large	25.6	23.1	65.8	8.76	235
A medium	25.9	21.3	66.6	8.75	247
A pullet	25.9	21.8	68.0	8.67	241
Necessary difference, 5% level	0.3	1.6[1]	1.8	0.09[1]	24[1]
Averages for eggs produced in five areas					
A medium	26.0	21.3	67.0	8.73	247
B	26.2	22.4	69.5	8.67	247
C	26.6	24.6	69.7	8.59	243
Necessary difference, 5% level	0.3	1.4	1.7	0.13[1]	20[1]

Difference not significant.

TABLE II

RELATION BETWEEN AREA IN WHICH EGGS ARE PRODUCED, SOLIDS CONTENT, AND QUALITY OF
EGG POWDER PRODUCED DURING A SEVEN MONTH PERIOD

Area of production	Solids content, %	Quality of powder			
		Fluorescence value	Potassium chloride value, %	pH	Foaming volume, ml.
Averages for Grade A eggs					
Vancouver	25.7	23.3	64.9	8.75	242
Edmonton	25.6	21.4	68.5	8.74	252
Winnipeg	25.5	22.4	66.2	8.78	242
London	25.7	22.5	66.7	8.73	239
Belleville	25.8	20.4	68.2	8.74	250
Three Rivers	26.0	21.9	65.4	8.67	236
Necessary difference, 5% level	0.4[1]	2.2[1]	2.6	0.13[1]	33[1]
Averages for Grades A medium, B, and C eggs					
Edmonton	26.7	23.4	70.8	8.60	249
Winnipeg	26.0	22.8	67.5	8.78	253
London	26.1	21.9	69.3	8.70	238
Belleville	25.8	21.3	67.5	8.66	251
Three Rivers	26.5	23.8	67.8	8.59	238
Necessary difference, 5% level	0.4	1.9[1]	2.2	• 0.17[1]	25[1]

[1] *Difference not significant.*

The effect of area of production on the solids content was given further consideration (Table IV). This table indicates a greater proportion of eggs of low solids content from Western Canada. However, Grade *C* eggs from Edmonton had high solids contents (average, 27.8%), thus accounting for the high value noted in the second part of Table II.

The only differential behaviour of significance was noted for eggs from Belleville. Grade *C* eggs from this area had, on the average, lower solids content than Grade *B* eggs, which in turn had a lower solids than Grade *A* mediums. The average values were: Grade *C*, 25.5%; Grade *B*, 25.8%; Grade *A* medium, 26.0%. No explanation can be offered to show why these results do not conform to other data presented in this paper or to everyday experience.

Calculations on powder yield in relation to solids entering the drier showed no differences ·in drying characteristics as a result of differences in grade, area, or time of production. The average recovery on the model drier was 92%. Although the distribution curve was slightly skewed, the standard deviation was calculated as 6%, which gives some indication of the variability observed.

TABLE III

RELATION BETWEEN MONTH IN WHICH EGGS ARE PRODUCED, SOLIDS CONTENT, AND QUALITY
OF THE POWDER PRODUCED

Month of production, 1944–5	Solids content, %	Quality of the powder			
		Fluorescence value	Potassium chloride value, %	pH	Foaming volume, ml.
Averages for Grade A eggs produced in six areas					
December[1]	25.4	21.9	61.2	8.96	223
January	25.6	24.0	64.0	8.88	234
February	25.6	24.8	66.4	8.74	219
March	25.7	21.5	69.3	8.73	265
April	25.9	22.5	65.9	8.74	237
May	25.8	21.8	65.6	8.76	232
June	26.2	21.6	71.7	8.78	285
July	26.0	17.9	66.7	8.51	235
Necessary difference, 5% level	0.5	2.4	2.8	0.14	36
Averages for Grades A medium, B, and C eggs produced in five areas					
December[1]	26.2	22.3	61.6	8.88	223
January	26.2	24.9	64.9	8.80	234
February	26.2	26.1	66.1	8.66	220
March	26.1	20.6	69.0	8.72	251
April	26.2	22.1	69.8	8.77	248
May	26.3	22.9	69.3	8.70	244
June	26.5	22.3	72.7	8.73	282
July	26.5	19.4	68.3	8.26	239
Necessary difference, 5% level	0.4	2.2	2.6	0.20	30

[1] *Eggs from only four areas available in December. Therefore these measurements not included in analysis of variance.*

TABLE IV

VARIATION IN SOLIDS CONTENT OF VARIOUS GRADES OF EGGS FROM VARIOUS DISTRICTS SAMPLED
DURING AN EIGHT MONTH PERIOD

Grade of eggs	Solids content, %			Area and number of samples greater or less than standard deviation											
				Greater						Less					
	Mean	S.d.	Range	V.	E.	W.	L.	B.	T.R.	V.	E.	W.	L.	B.	T.R.
Production from six areas															
Grade A large	25 6	0.5	24.5 – 26.3	1	1	1	1	0	3	1	3	1	1	1	0
Grade A medium and pullet	25.9	0 5	24.6 – 27.3	0	1	0	2	1	3	5	1	0	3	1	0
Production from five areas															
Grade B	26.2	0.4	25.0 – 27.5	—	0	1	0	1	0	—	2	0	0	0	1
Grade C	26.7	1.0	25.5 – 29.9	—	2	0	0	0	0	—	0	0	0	2	0

The data in Table IV indicated that, in spite of fairly constant behaviour during the drying operation, variation in production is possible because of differences in solids content of the eggs. In Canada, only eggs of *B* grade or better are dried, thereby making possible variation in recovered solids of between 24.5 and 27.5 lb. per 100 lb. of liquid egg introduced into the drier. Since the quantity of liquid egg dried in one day in a commercial plant greatly exceeds the size of the samples studied here, some reduction in this variation is likely. If variations of the magnitude of the standard deviations (Grades *A* and *B* eggs, Table IV) are encountered in commercial practice, 100 lb. melange may produce between 25.1 and 26.6 lb. of powder. That is, a plant drying 40,000 lb. of liquid egg per day may recover between 10,040 and 10,640 lb. of solids. These values are more extreme than, but support, the 400 lb. differences reported from some commercial plants producing about this quantity of powder.

Quality of the Powder Produced

Neither grade of eggs nor area of production had a significant effect on the pH and foaming volume values of the powder produced, but month of production appeared to affect all quality measures (Tables I, II, and III). The use of Grade *C* eggs resulted in powders with increased fluorescence values. The high fluorescence value noted for Grade *A* large eggs was attributable to the high values for this grade of eggs from the Vancouver area. Powders produced from Grade *A* pullet eggs had higher potassium chloride values than powder from Grade *A* large eggs, and Grades *B* and *C* eggs produced powder significantly better in this quality attribute than Grade *A* mediums. Powder from Grade *A* eggs from Edmonton and Belleville areas had higher potassium chloride values than powder from similar eggs produced in the Vancouver and Three Rivers areas. The comparison of *B* and *C* with *A* medium eggs further supported the high potassium chloride values for eggs from the Edmonton area.

Time of production affected all quality attributes of the powder (Table III). Fluorescence values were high in February and decreased thereafter, while potassium chloride values progressively increased until June and decreased markedly in July (time of first moult). A regular decrease in pH was observed throughout the period studied. Foaming volume values were greatest in March and June. Since there were changes in quality with month of production, which may have been the results of climatic conditions, these changes were studied further by calculation of the correlation coefficients between quality attributes and climatic condition.

Correlations Between Time, Climatic Conditions, and Quality

Correlation of the various measurements for Grade *A* eggs or powder from Grade *A* eggs with time of production is shown in Table V. Average values for solids content and potassium chloride value and foaming value of the powder increased as the season of production progressed, while average fluorescence value and pH of the powder decreased. The only relations

attaining significance were those between time of production and solids content, and time of production and pH. Considered independently of time effects, positive correlations were observed between average solids content and average fluorescence, potassium chloride and pH values of the powder. However, these relations failed to attain significance.

TABLE V

CORRELATION COEFFICIENTS BETWEEN TIME OF PRODUCTION, AVERAGE
SOLIDS CONTENT OF LIQUID EGG, AND AVERAGE VALUES FOR
QUALITY MEASUREMENTS ON EGG POWDER

Items correlated	Correlation coefficient
Time correlated with averages for: (6 degrees of freedom)	
Solids content of liquid eggs	.886**
Fluorescence value of powder	−.704*
Potassium chloride value of powder	.656
pH of reconstituted powder	−.799*
Foaming volume for powder	.456
Correlation of solids content with averages for quality measures independent of time effects: (5 degrees of freedom)	
Fluorescence value of powder	.417
Potassium chloride value of powder	.558
pH of reconstituted powder	.375
Foaming volume for powder	.077

* 5% point of statistical significance, $r = \pm.707$.
** 1% point of statistical significance, $r = \pm.834$.

When considering the effects of climatic conditions (dry bulb temperature, sunshine, rainfall) it was believed that the average condition occurring during the first 15 days of the month might be correlated with the various measures, if the quality was affected by the climate to which the egg is exposed just after laying. Conversely, if climate were affecting egg quality prior to laying, the average condition occurring during the last 15 days of the preceding month might be correlated with the measures. To make the comparison, correlation coefficients were calculated independently of time and area. Only one relation attained significance (5% level) and since there were 30 comparisons this might be expected to occur by chance (Table VI). It was thought possible that significant relations might be observed if the data were broken down for periods from January to March and April to June, i.e., for periods when hens are sheltered or outside. However, even with this breakdown none of the coefficients became significant.

In general, the failure of the correlations to attain significance for any factor other than time indicates that the age of the hen may be the most important single factor responsible for the changes noted in the solids content

TABLE VI

CORRELATION COEFFICIENTS RELATING CLIMATIC CONDITIONS TO THE AVERAGE SOLIDS CONTENT
OF GRADE A EGGS COLLECTED ON THE 15TH OF EACH MONTH FROM JANUARY TO JUNE
AND TO THE QUALITY OF THE POWDER PRODUCED (INDEPENDENT OF TIME
AND AREA[1])

Average condition during the first 15 days of the months correlated with:	Correlation coefficient (15 degrees of freedom)		
	Dry bulb temperature	Sunshine	Rainfall
Liquid egg			
Solids content	−.313	−.160	.354
Powdered egg			
Fluorescence value	.068	−.411	.196
Potassium chloride value	195	.439	.360
pH	−.143	.280	.096
Foaming volume	.259	−.483*	−.072
Average condition during the last 15 days of the preceding months correlated with:			
Liquid egg			
Solids content	.187	−.145	.282
Powdered egg			
Fluorescence value	.008	−.345	.373
Potassium chloride value	− 030	−.353	.336
pH	− 021	.147	−.040
Foaming volume	− 045	−.306	−.255

[1]Only samples taken from January to June and eggs from Three Rivers, Winnipeg, Edmonton, and Vancouver were considered in this treatment.
* 5% point of statistical significance, r = ±.482.

of the liquid egg and in the quality of the powder produced.   The differences noted for various areas may be attributable to local variations in feeding practice.

Acknowledgments

References

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis. 5th ed. A.O.A.C., Washington, D.C. 1940.
2. DEPARTMENT OF AGRICULTURE, CANADA. Regulations respecting the grading, packing and marking of eggs. Ottawa. 1940.
3. HENDERSON, W., NAROD, M., COOK, G., and LLOYD, E. A. Poultry Sci. 20 : 462-463. 1941.
4. HUNTER, J. A., VAN WAGENEN, A., and HALL, G. O. Poultry Sci. 13 : 314-315. 1934.
5. HUNTER, J. A., VAN WAGENEN, A., and HALL, G. O. Poultry Sci. 15 : 115-118. 1936.
6. NATIONAL RESEARCH COUNCIL AND THE SCIENCE SERVICE OF THE DEPARTMENT OF AGRICULTURE, CANADA. The official methods used in assessing quality in Canadian dried whole egg powder. Ottawa. 28 Feb. 1944,
7. PAULHUS, N. G. and GWIN, J. M. Poultry Sci. 19 : 357-358. 1940.
8. PEARCE, J. A., THISTLE, M. W., and REID, M. Can. J. Research, D, 21 : 341-347. 1943.
9. REID, M. and PEARCE, J. A. Can. J. Research F, 23 : 239-242. 1945.
10. TESSIER, H., MARIER, J. R., and PEARCE, J. A. In preparation.
11. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E. Can. J. Research, D, 21 : 1-7. 1943.
12. WILHELM, L. A. and HEIMAN, V. Poultry Sci. 16 : 362. 1937.
13. WOODCOCK, A. H. Can. J. Research, D, 21 : 90-97. 1943.
14. WOODCOCK, A. H. and TESSIER, H. Can. J. Research, A, 21 : 75-78. 1943.

DRIED WHOLE EGG POWDER

XXI. PASTEURIZATION OF LIQUID EGG AND ITS EFFECT ON QUALITY OF THE POWDER[1]

By N. E. Gibbons[2], C. O. Fulton[3], and Margaret Reid[4]

Abstract

Heating liquid egg to 60°C. reduced the viable bacterial count 85 to 95% but had less effect on the coliform organisms. Holding the liquid at 60°C. for 30 min. reduced the viable count 98 to 99%, and destroyed the coliforms, *Salmonella*, and *Staphylococci*. Quality tests indicated no difference between powders prepared from heated and unheated melange either originally or after storage. Tests with a laboratory flash pasteurizer indicated that a considerable reduction in the number of total viable and coliform organisms occurred when liquid egg was heated from 22° to 60°C., the total time in the pasteurizer being approximately one minute.

Introduction

The presence of organisms of the *Salmonella* group (4) and coagulase positive *Staphylococci** in dried egg powder introduced a public health hazard that at one time was viewed with some alarm. Previous studies indicated that in the interests of quality the temperature of drying should be as low as possible and the powder should be cooled as rapidly as possible (12). Both of these recommendations favoured the survival of bacteria during the drying process (3). The present studies were undertaken to determine the feasibility of destroying possible pathogens by heat treating liquid egg and to evaluate the effect of this treatment on the keeping quality of the powder.

Although the principle of heating liquid egg to facilitate drying was well known, little information was available on the heat treatment of liquid egg with the object of destroying bacteria. It has been stated that broken out duck eggs may be pasteurized by adding sodium citrate, heating to 65°C. for 20 min., and cooling rapidly (10). In a criticism of this method it was claimed that the heating period of 20 min. was not always sufficient to kill *Salmonella* (11). Recently some work on hens' egg melange has been done in the United States (5).†

Materials and Processing Methods

Preliminary tests indicated that fresh egg liquid could be heated to 52° and 59°C. (126° to 138°F.) for periods up to 12 hr., with a reduction in the bacterial content, but without affecting the fluorescence value of the powder prepared from it. Fresh melange could be heated to 66°C. (151°F.) and held 30 min. with no evidence of coagulation, provided it was stirred vigorously. There was some coagulation when the temperature was raised to 68°C. (154°F.). It was not possible to obtain these temperatures in the commercial trials with the equipment available as the temperature differentials necessary were so high that coagulation occurred on the heating coils; the highest practical temperature was 60°C. (140°F.).

Liquid egg was divided into two portions, one of which was inoculated with *Escherichia coli*, and the other with an organism resembling *Streptococcus faecalis*. Portions of each lot were heated in 5 to 10 min. to 55°, 57°, and 60°C. (131°, 135 5°, and 140°F.). After one-half, one, two, three, and four hours, samples were removed for bacteriological analysis and for drying. The samples for drying (100 ml.) were frozen immediately at −40°C. and dried in a vacuum over calcium chloride as soon as possible. The powders were stored at −40°C. until all had been prepared. Fluorescence and potassium chloride were then determined and the powder stored 21 days at 37°C. when the fluorescence was again measured.

Based on the results of these tests, further experiments were made with mixtures of *E. coli*, *E. freundii*, and *Aerobacter aerogenes*, of *Salmonella*, and of hemolytic *Staphylococci* and *Streptococci*.

For the first commercial trial, 2000 lb. of liquid egg prepared from frozen blocks was used. The drier and all tanks and lines had been cleaned some hours previously and filled with hypochlorite solutions and rinsed with hot water. The melange was heated to 60°C. in a cream forewarmer, consisting of an open uninsulated metal tank with a rotating helix through which hot water was circulated at 82°C. (180°F.). The mass was heated from 8° to 60°C. in 54 min. This time could probably have been shortened but the temperature at which the egg might coagulate on the coils was not known. After holding the liquid 30 min. at a temperature of 59° to 60°C., it was pumped into an insulated holding tank and fed as needed through a line filter into a small ballast tank near the drier. All of the liquid was dried 2.5 hr. after pasteurizing. The temperature of the melange and outlet air temperature of the drier are given in Fig. 1. Samples of powder were collected 10, 85, and 145 min. after drying began and immediately cooled. As soon as the heated egg was dried, the drying of unheated liquid from the same master batch was resumed and after 30 min. of operation a sample of powder was collected as a control. All samples were analysed bacteriologically and chemically at once and after storage for three weeks at 37°C.

In a second trial, five lots of 2000 lb. each were processed. The melange was prepared the day before, cooled, and held at 4°C. (39°F.) overnight. The

holding temperatures and times are given in Table III.   Lot *B* was raised to temperature more rapidly than *A*.   The control powder (*F*) was prepared from the same master batch of melange immediately after the others were dried.   Throughout the trials the temperature of the water entering the coils
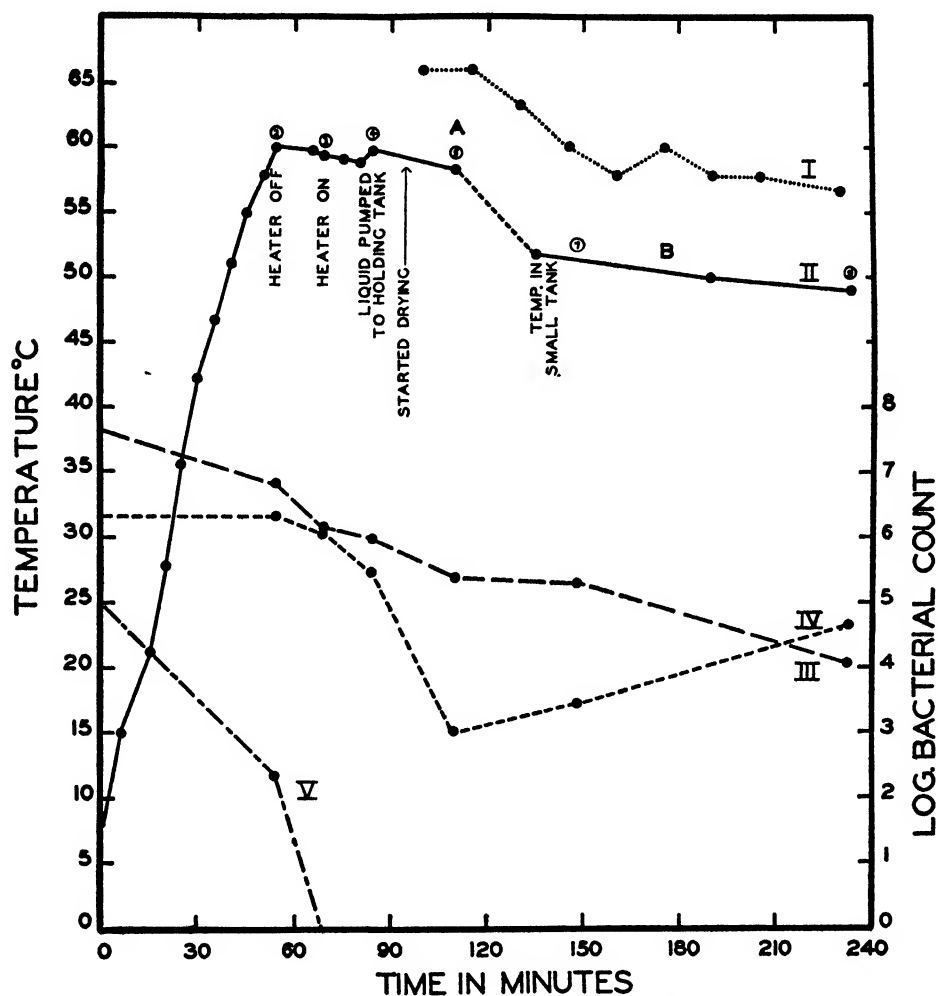

FIG. 1. *Data regarding first commercial run.   I—outlet air temperature of drier;   II—temperature of liquid egg;   III—viable bacterial count at 37°C.;   IV—viable bacterial count at 55°C.;   V—coliform content;   1, 2, etc.—samples of liquid egg taken;   A, B—samples of powder taken.*

was between 81° and 86°C. (178° to 187°F.).   The air inlet temperature to the drier was 96°C.; the outlet temperature varied between 57° and 60°C. On leaving the drier the powder was cooled in a ramshorn with air at 5°C. Approximately 10 lb. from the first of each run was excluded from the test material.   Samples for analysis were taken as the powder was being sifted into barrels.

Between lots, all lines and tanks, with the exception of the ballast tank, were washed with hot water and steam. The ballast tank was merely washed with hot water to allow continuous operation of the drier.

The powder was stored at 16°C. (60°F.) and packaged after five days. At that time 14 lb. of test material was removed from each lot by taking small amounts throughout the packaging operation. This was stored at −1.1°C. (30°F.) for six days, then sealed in cans and stored at 32.2°C. (90°F.) for periods up to four weeks, at 26.7°C. (80°F.) up to 12 weeks, and at 21.1° (70°F.) and 15.6°C. (60°F.) up to eight months. The remainder of the powder was shipped overseas in the usual 14-lb. packages.

Since there are several disadvantages to vat pasteurization, a small experimental unit was constructed to explore the possibility of flash pasteurizing the liquid egg immediately before it entered the drier. Three glass tubes 80 cm. long with inside diam. of 3 mm. were enclosed in a water jacket made of glass tubing (41 mm. I.D.).

Water was pumped through the jacket from a constant temperature bath by means of a centrifugal pump. The egg was forced through the tubes (total capacity approximately 17 ml.) at the rate of about 20 ml. per min. by a constant volume delivery pump. Some liquid egg pasteurized in this apparatus was pumped directly into a laboratory spray drier (14).

Analytical Methods

Viable bacterial counts were made on proteose-peptone–tryptone agar after incubating at 37°C. (98°F.) for three days. In the first commercial trial, duplicate plates were also incubated at 55°C. (131°F.). The presence of coliform organisms was detected by inoculating appropriate dilutions into five tubes of brilliant-green bile broth. Gas production was checked after 24 and 48 hr. incubation at 37°C. and the most probable number determined (1).

Powder quality was evaluated by fluorescence value (8), potassium chloride value (9), moisture (9), and palatability (8).

Results

Effect of Pasteurization on Bacterial Content

The results of several preliminary experiments are summarized in Table I. At 60°C. the viable count reached a minimum and all coliforms had been killed in 30 min. At 57.5°C. this was not accomplished for at least three hours. Mixtures of at least two strains each of *E. coli*, *E. freundii*, and *A. aerogenes* and of five types of *Salmonella* were destroyed in 25 min. at 60°C. Six strains of α-toxin producing, coagulase-positive *Staphylococci* were killed in 15 min. at 60°C. and four strains of Group A *Streptococci* were killed in 10 min. at 60°C., although 20 min. was taken to attain the desired temperature. It would seem therefore that 60°C. for 30 min. is the shortest time possible for vat pasteurization.

TABLE I

EFFECT OF HEATING LIQUID EGG ON THE BACTERIAL CONTENT

Organism added	Approx time to attain pasteurization temp, min	Pasteurization temp, °C	Holding time at pasteurization temp min	Log no organisms per ml			
				Initial		After heating	
				Viable	Coliform M P N †	Viable	Coliform M P N
E coli*	8	60	15	7 57	6 25	4 00	0 30
			30			3 45	0
			60			3 34	0
	5	57 5	30	7 73	7 85	4 22	1 34
			60			3 63	1 23
			120			3 48	1 34
			240			3 30	30
	5	55 0	30	7 85	6 25	5 74	3 30
			120			4 04	2 20
			240			3 59	2 26
E coli** } E freundii } A aerogenes }	4	60	10	7 13		3 73	
			20			1 18	
			25			0	
Salmonella spp **	4	60	10	7 26		3 04	
			20			1 70	
			25			0	
Staphylococci** α-toxin coagulase +	20	60	0	7 50		2 60	
			15			0	
Streptococci** Group A	20	60	10	6 90		0	
S faecalis*	10	60	30	4 70		3 45	
			60			3 41	
			120			3 32	

†M.P N —Most probable number of organisms.
*Added to melange containing other organisms
**Added to melange containing < 10 other organisms per ml.

The first commercial trial was based on these results   The pertinent data and the decrease in bacterial content are shown in Fig 1

The high initial viable count was attributed to thawing frozen melange with warm water and failing to cool it promptly   It served admirably for the purpose of the test.   The viable count at 37°C had decreased to about one-sixth of the original by the time the temperature of the liquid reached 60°C. and at the end of the 30 min holding time there was a 98% reduction. The reduction in count continued in the liquid awaiting drying, and at the final sampling was 99 97%.

The number of coliform organisms decreased from 92,000 per ml. initially to 220 per ml. by the time the temperature of the liquid had reached 60°C., and none could be detected in 10-ml. amounts after 15 min heating.

Organisms capable of growing on plates incubated at 55°C. were reduced to only 15% of the original number at the end of the pasteurizing period   The number continued to decrease for a short time afterwards, but as the temperature of the melange approached 55°C. began to increase again. Since none

of these organisms is pathogenic, they are of little significance.   However, their behaviour serves to point out some of the dangers inherent in batch processes and to emphasize the fact that the liquid should be dried as rapidly as possible after heating.

The powder prepared from the pasteurized liquid contained only a small fraction of the number of viable bacteria present in that prepared from untreated egg (Table II).

TABLE II

QUALITY MEASUREMENTS AND BACTERIAL CONTENT OF POWDERS PRODUCED FROM PASTEURIZED AND UNPASTEURIZED LIQUID EGG (FIRST COMMERCIAL TRIAL)

| | Moisture % | Fluorescence value | | Palata-bility ratings | Potassium chloride value | Viable bacterial count, log. no. per gm. | |
		Initial	After 3 weeks at 37°C.			37°C.	32°C.
Pasteurized*							
G	2.77	13.5	43.3	7.5	68.2	4.71	4.82
H	3.05	14.0	42.7	8.0	72.5	4.64	4.62
I	2.80	15.0	40.0	8.5	70.5	4.54	4.58
J Unpasteurized	4.16	14.0	46.4	8.5	73.1	6.88	6.93

*Collected 10, 85, and 145 min. respectively after drying began.

TABLE III

EXPERIMENTAL DATA ON SECOND COMMERCIAL PASTEURIZATION EXPERIMENT

Lot	Holding temp., °C.	Time to attain temperature, min.	Holding time, min.	Moisture content of powder, %
A	60	69	30	3.75
B	60	36	27	3.60
C	60	48	0	3.68
D	57	36	10	4.02
E	57	64	0	4.16
F (control)	—	—	—	3.77

In the second commercial trial the heating-up period was the most destructive to the bacterial population, but within the limits studied the time taken to reach temperature had little effect (Table IV).   The time of holding had little effect on the final number of organisms.   The temperature attained was most important in determining the viable count in the liquid.

The heating-up period (Table IV) was as destructive to the bacteria as the whole pasteurizing period in the previous experiment (Table II). In the

control powder (*F*) the percentage reduction due to drying alone was as great as that obtained by both pasteurizing and drying. Although such high percentage reductions due to drying alone have been reported (7), they are not usual (2, 3) and the extent of reduction probably depends on the particular flora of the melange being dried. The data for coliform organisms are unfortunately not complete; nevertheless, the indications are that at these temperatures a holding period is necessary to eliminate these organisms from the melange (Table IV). The high coliform content of the powder produced

TABLE IV

LOGARITHM OF NUMBER OF VIABLE ORGANISMS GROWING AT 37°C. AND OF MOST
PROBABLE NUMBER OF COLIFORMS AND *E. coli* IN EGG MELANGE
AND POWDER IN SECOND COMMERCIAL EXPERIMENT

Sample*	Log. viable count, 37°C.					
	A**	B	C	D	E	F
1	6.39	6.53	6 60	6.28	6 83	—
2	4.56	4.46	4.47	4.98	4.93	—
3	4.34	4 43	—	4.70	—	—
4	4.26	4.14	4 24	4.57	4.60	—
5	4.79	4 82	4.99	5.20	5.31	5.17

Sample*	Coliforms			E. coli		
	A	C	E	A	C	E
1	4 29	—	4 59	3 99	—	4.08
2	—	2 10	2.54	—	2.10	2 41
3	1.78	—	—	1.54	—	—
4	—	—	1.75	—	—	1.08
5	2 02	0	0.48	1.34	—	0

*Sample 1—before heating, 2—on reaching temperature, 3—at end of holding period, 4—from ballast tank, 5—powder.
**For treatment see Table III.

from the first run (*A*) was no doubt caused by contamination. Later tests indicated that the high pressure pump may have been the source of this contamination.

The results of the initial experiments with the flash pasteurizer are shown in Table V. By appropriately adjusting the temperature of the water jacket, the temperature of the effluent egg was varied by one-degree intervals between 55 and 62° C. The original melange contained 625,000 organisms per ml. and, with 1-ml. amounts, 10 of ten tubes were positive for coliform organisms. The initial temperature was 20° to 22°C. A 99% reduction in the viable count was accomplished with an effluent temperature of 57°C. and the reduction increased as the temperature increased. With the dilutions used, little information was obtained about the reduction in numbers of coliform

organisms; no reduction was apparent until a temperature of 62°C. was reached. However, the liquid egg leaving the pasteurizer at 61°C. was slightly thick, so 60°C. was regarded as the highest working temperature with this equipment.

TABLE V

EFFECT OF TEMPERATURE ON BACTERIAL CONTENT OF
FLASH PASTEURIZED MELANGE

Temp. egg at outlet, °C.	Viable count, no. per ml.	Coliform: no. of 10 tubes positive
55	2600	10
56	22,000	10
57	1300	10
58	590	10
59	750	10
60	150	10
61	50	9
62	25	1
Original melange	625,000	10

In a later trial the pasteurizer was set up in conjunction with the laboratory spray drier and three lots of powder dried (Table VI). Lot K was put through the pasteurizer, cooled immediately, and later put through the drier. Lot L was pasteurized and pumped directly into the drier without cooling. Lot M

TABLE VI

EFFECT OF FLASH PASTEURIZATION ON THE BACTERIAL CONTENT OF EGG POWDER AND ON
THE QUALITY INITIALLY AND AFTER STORAGE FOR
THREE WEEKS AT 37°C.

Lot*	Viable count, gm.	Coli, M.P.N./ gm.	Mois- ture, %	Fluorescence values		Potassium chloride value		Palat- ability
				Initial	After storage	Initial	After storage	Initial
K	10,000	1000	2.63	21.9	50.9	65.0	40.0	7.8
L	8000	0	2.25	21.5	52 0	71.0	43 0	8.8
M	34,000	5000	3.98	22.0	60.6	64.6	38 0	8.3

*See text for treatments.

(control) was put through the cold pasteurizer. In lots K and L the water jacket was maintained at a temperature of 62.5°C. and the egg liquid at the outlet ranged from 59.5° to 60°C.

The original melange contained 1,700,000 organisms per ml. The melange of lot K had a viable count of 140,000 per ml. after pasteurization (92% reduction). The data on the powders (Table VI) indicated that pasteurization

followed immediately by drying (lot *L*) is more effective than cooling the pasteurized liquid and drying later. However, the results are not conclusive and further work is necessary with more efficient heat exchangers.

Effect of Pasteurization on Powder Quality

The early experiments indicated that heating liquid egg for periods up to four hours did not increase the development of fluorescence during storage of the resulting powder (Table VII); the indications were that the longer heating periods retarded the development of fluorescence.

TABLE VII

EFFECT OF STORAGE FOR 21 DAYS AT 37°C. ON FLUORESCENCE VALUES OF POWDER PREPARED FROM UNHEATED AND HEATED LIQUID

Holding time, hr.	Fluorescence values	
	Initial	After storage
0	12.5*	29.8*
0 5	11.9	28.8
1	11.9	29.2
2	12.1	28.7
3	12.1	26.6
4	11.8	25.0
Necessary difference	1.1	2.7

Means of three trials at 55°, 57.5°, and 60°C. The difference between them was not significant. Moisture content of all powders, 2.58 ± 0.10%.

In the first commercial trial no significant differences between the powder prepared from treated and untreated melange could be detected initially by any of the measures of quality used (Table II). After storage at 36.7°C. for periods up to three weeks the fluorescence values of the pasteurized material were slightly less than those of the controls (Table II). This was attributed in part to the higher moisture content of the latter (13). As in the early experiment there was an indication that the longer the liquid was kept hot the slower the development of fluorescence. This possibly indicates a destruction of enzymes.

The lower moisture content of the powder prepared from the heated melange is interesting. As may be seen from Fig. 1 the air outlet temperature was very high at the beginning owing to inexperience in drying hot liquid. It was necessary to reduce the heat input into the incoming air by one-half to bring the outlet temperature to the usual 135°F. As the same nozzle and the same pump pressure (2800 lb. per sq. in.) were used, no difference was observed in the amount of powder dried.

The moisture contents of the powder prepared in the second commercial trial were more comparable (Table III). The changes in fluorescence and potassium chloride values during storage at 32.2°, 26.7°, 21.1°, and 15.0°C.

of powders prepared from pasteurized melange and the control powder are shown in Fig. 2. Fluorescence measurements showed no difference between the treated and untreated material. Although average values only are shown in the figures, the potassium chloride values of the powders from unheated egg and that heated to 57°C. were slightly higher than those from egg heated to 60°C. This difference was apparent during most of the storage period but was not always noticeable at the end.
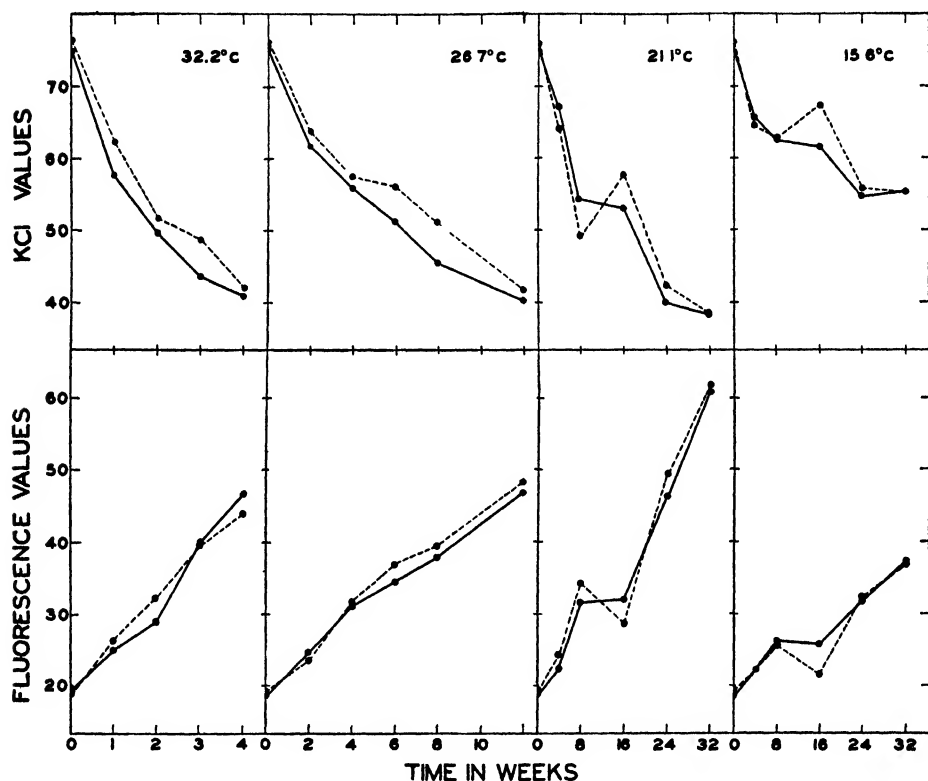
FIG. 2. *Changes in fluorescence and potassium chloride values of pasteurized and unpasteurized egg powders on storage. Treatment of liquid egg as in Table III. Solid line—unheated liquid. Broken line—mean value for five lots of powder from pasteurized liquid.*

The powder shipped overseas was tested there by a number of other tests (Haenni solubility, foam volume, creaming, flavour, and pH), with insignificant differences between the pasteurized powders and the control (6). It was remarked that the pasteurized powder was caked to some degree and resembled material that had been lightly compressed and then crumbled. This had been noted when packing the material but the lumps were easily broken up.

Samples of lots *A* and *F* were stored overseas at 37°C. for 10 weeks with no difference·between the two in flavour, foam volume, creaming, and Haenni solubility.

With the exception therefore of a tendency to pack and a slightly lower solubility there was little or no difference in the original and keeping properties of egg powders prepared from untreated and vat pasteurized melange.

Panels of six persons passed judgment on scrambles prepared from all of the powders of the second commercial experiment before and after storage. Considering the variation between persons, there was little or no difference in the ratings given the pasteurized powders as compared with the controls. Treated and untreated powders deteriorated at practically the same rate.

The results of the initial quality tests on the powders prepared with the flash pasteurizer are shown in Table VI. There was little difference between the control (lot M) and the treated material. The deterioration during storage at 37°C. for three weeks was practically the same for all three powders, considering the higher moisture content of the control.

Commercial apparatus for flash pasteurization was not available for further work. However, such apparatus operating with lower temperature differentials and more turbulent flow might allow higher temperatures to be used without coagulation. Regulating the flow to drier capacity would eliminate the accumulation and holding of hot liquid, and conserve the heat introduced into the liquid.

Acknowledgments

References

1. AMERICAN PUBLIC HEALTH ASSOCIATION. Standard methods for the examination of dairy products. 8th ed. New York, 1941.
2. BARTRAM, M. T. J. Bact. 45: 48. 1943.
3. GIBBONS, N. E. and FULTON, C. O. Can. J. Research, D, 21 : 332-339. 1943.
4. GIBBONS, N. E. and MOORE, R. L. Can. J. Research, F, 22 : 48-57. 1944.
5. GRECO, P. A. Iowa State Coll. J. Sci. 19 : 18-19. 1944.
6. HAWTHORNE, J. R. Personal communication.
7. JOHNS, C. K. Sci. Agr. 24 : 373-382. 1944.
8. PEARCE, J. A. and THISTLE, M. W. Can. J. Research, D, 20 : 276-282. 1942.
9. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E. Can. J. Research, D, 21 : 1-7. 1943.
10. VAN OIGEN, C. F. Tijdschr. Diergeneesk. 67 : 280-300. 1940.
11. WEDEMANN, W. Z. Fleisch-Milchhyg. 50 : 277-279. 1940.
12. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 194-202. 1943.
13. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 211-222. 1943.
14. WOODCOCK, A. H. and TESSIER, H. Can. J. Research, A, 21 : 75-78. 1943.

FLUORESCENCE SPECTRA OF EXTRACTS OF
DRIED WHOLE EGG POWDER*

Jesse A. Pearce and W. A. Bryce

Division of Applied Biology.
National Research Laboratories,
Ottawa, Canada

INTRODUCTION

The fluorescence of saline extracts of defatted egg powder is related to the eating quality (11) and increase in the fluorescence value is a good measure of deterioration during storage (10, 17). Further work both here (7,8,18) and elsewhere (1,6,17) has led to the belief that the deterioration in egg powder was related to the protein and carbohydrate fractions. Therefore, information about reactions between sugars and proteins was sought.

An excellent description of reactions between sugar and amino acids has been presented by Enders (2). This work suggests a mechanism for such a reaction and indicates that the reaction product contains a five-membered heterocyclic ring composed of four carbon atoms and a nitrogen atom. These reactions have been summarized elsewhere (9).

During previous work, it was noted that a yellow, fluorescing substance was extracted from chloroform defatted egg powder (7). An attempt was made to isolate some of the fluorescing substance, but, before this work had been carried very far, the difficulty of separating and isolating the fluorescing fractions was apparent. Since the substances fluoresced, it was believed that a technique similar to that used to identify carcinogenic substances (4) might be applicable. The Coleman photofluorometer was modified and used to make crude estimations of the fluorescence spectra of egg powder extracts.

While some work has been done to show a relation between fluorescence and the component parts of a molecule, little information was available that could be applied to the present problem (13, chap. XIV). Therefore, the fluorescence of a number of chemicals was examined to provide a background of information for work on egg extracts.

The present paper describes the fluorescence spectra of egg powder extracts and compares them to spectra of various chemicals and of the reaction products of various amino acids and sugar-like substances. In addition, absorption spectra of some of the fluorescing materials are described.

MATERIALS AND METHODS

The dried egg products used were materials available from storage studies in progress in the laboratory. Their quality was assessed by the standard fluorescence method (12). The chemicals were either the best available from commercial sources or were purified products obtained from other investigations in these laboratories. Solvents used for the various chemicals, chloroform, alcohol, ether and water, were redistilled in the laboratory. For the extraction of fluorescing

---

material from egg powder, the organic solvents were used in the anhydrous state.

The Coleman photofluorometer was modified for use as a 'fluorospectrometer' by substituting, for filter PC-1, filters similar to those used in a colour-measuring device (19). A group of nine filters was used to separate the visible portion of the spectrum into bands about 30 mμ in width. All results presented here are expressed in terms of the principal wave lengths of these bands. Unfortunately, during a portion of the investigation a faded set of filters was used and no blank correction was made for solvents. However, sufficient reference curves were available to permit conclusions of value.

Since the transmission in the various filters was not of equal intensity, a correction factor was necessary (Table I). The table also shows that the band with a principal wave length of 412 mμ transmitted too much light to permit a reading on the scale; possibly some incident light, since the B_1 filter transmits light up to about 410 mμ (3). Comparison of the transmission curve for filter PC-1 and a fluorescence curve for a pyrrole solution (Fig. 1) indicated that light around 412 mμ was not transmitted to the photocell in the ordinary fluorescence measurement.

To maintain the instrument at the same setting during a determination, it was checked frequently using filters B_1 and PC-1 and standard quinine sulphate (0.2 γ per ml.). Under these conditions, the fluorometer reading was set at 50. Coleman cuvettes transmitted about 90% of the incident light at 360 mμ and were considered satisfactory. All fluorescence readings are given as units on the photofluorometer scale. Fluorescence determined using filter PC-1 was the standard of comparison for all materials studied. To provide fluorescing solutions within the range of the photofluorometer, the solutions were made up to a known fluorescence value rather than to predetermined concentrations.

The absorption curves presented here were determined using a Beckman Quartz Spectrophotometer.

RESULTS

Fluorescence Spectra of Chemicals and Various Reaction Products

The fluorescence spectra of various chemicals and reaction products of sugars and amino acids are given in Figs. 2 and 3 and in Table II. Substances with negligible fluorescence or no fluorescence are given in Appendix I.

Chemicals with a pyrrole ring had fluorescent spectra similar in nature (Fig. 2). However, carbazole had a spectrum identical with that of anthracene (Table II). Since anthracene is difficult to isolate (5, p.492) it is possible that one of the chemicals used contained the other as an impurity. It is more likely, however, that the spectrum is characteristic of condensed ring structures of this type. A number of other substances with a blue fluorescence had spectra similar to that of pyrrole, but the bands differed in intensity (Table II). Substances with other than a blue fluorescence had spectra differing completely from the foregoing (Fig. 2). The addition of a metallic ion caused a marked change in the fluorescence spectra (oleic acid and sodium oleate in Fig. 2).

It was believed possible that this technique might be used to differentiate between fluorescence in materials of a carbyhydrate nature (8) and other chemicals. Comparison of the spectrum for caramel (from heated sucrose) with spectra of pyrrole and dicyclopentadiene showed it to be different from eith of these two materials. It was not

TABLE I

SHOWING CORRECTIONS NECESSARY IN DETERMINATION OF
FLUORESCENCE SPECTRA OF PYRROLE (fluorescence with
filter PC-1, 60 photofluorometer units)

Principal wave length, mμ.	Fluorescence intensity		Correction for filters	Final reading	Relative intensity
	as read	less blank correction (alcohol)			
412	off scale	-	-	-	-
444	77.5	72.5	1.14	82.7	57.5
474	28.5	21.5	1.17	25.2	4.6
512	21.0	20.0	1.03	20.6	13.0
542	7.8	7.3	1.04	7.6	5.7
572	1.9	1.9	1.00	1.9	1.7
600	0.2	0.2	1.00	0.2	0.2
630	0	0	1.00	0	0
700	0	0	1.00	0	0
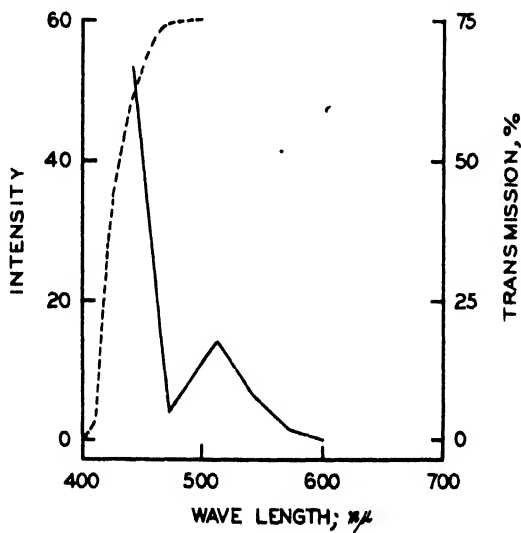
Fig. 1   Ultra-violet transmission of filter PC-1 (dotted line) compared with a portion of the fluorescence spectrum of pyrrole.

TABLE II

FLUORESCENCE SPECTRA OF VARIOUS SUBSTANCES, SIMILAR
TO PYRROLE IN THIS CHARACTERISTIC.   FLUORESCENCE WITH
FILTER PC-1, 50-55 (New filters and blank corrections
made)

Substance	Fluorescence intensity passing filter with principal wave length (mμ) of:					
	444	474	512	542	572	600 and higher
Anthracene and carbazole	90.5	0.6	4.1	0	0	0
Sodium salicylate	71.0	2.1	5.1	1.1	0.5	0
Oleic acid	57.4	1.8	10.2	3.7	1.0	0
Thiochrome	52.2	2.3	8.0	1.5	0.5	0
Pyrrole	48.2	3.4	10.0	5.0	1.4	0
Di-cyclopentadiene	41.7	2.5	12.8	4.2	1.5	0
Quinine sulphate at 45°F. and at 80°F.	39.4	3.4	11.3	4.2	1.0	0

TABLE III

EFFECT OF COOLING TO -112° F. FOLLOWED BY WARMING
TO ROOM TEMPERATURE ON AN ALCOHOL SOLUTION OF
NICOTINE (Initial fluorescence with PC-1, 61)

Sample	Fluorescence intensity with filters:						
	PC-1	444	474	512	542	572	600 and higher
Untreated	61	41.3	11.4	15.4	6.8	1.5	0
Cooled	76	61.8	4.0	18.4	8.9	1.5	0
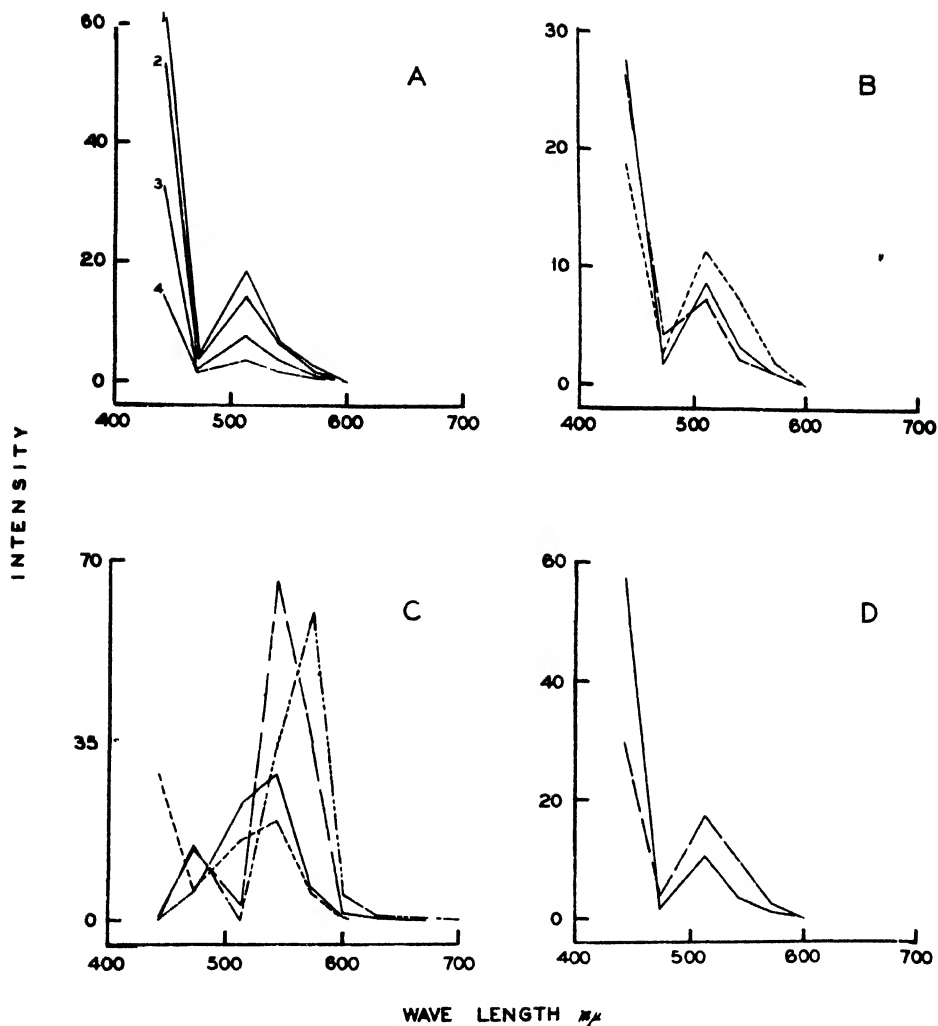
Fig. 2 Fluorescence spectra of various chemicals (all curves deter-
mined using new filters and with a blank correction).

A. 1. Nicotine; Fluorescence with PC-1, 70.
   2. Pyrrole and nicotine; Fluorescence with PC-1, 60.
   3. Pyrrole, nicotine and β-indolyl acetic acid; Fluores-
      cence with PC-1, 40.
   4. Pyrrole, nicotine and tryptophane; Fluorescence with
      PC-1, 20.

B. Pyrrole ————
   Di-cyclopentadiene —— —— ——
   Caramel ------
   Fluorescence with PC-1, 34.5

C. Sodium Fluorescein ————
   Acridine orange --------
   Eosin blue —— —— ——
   Acridine Red }
   Rhodamin G   } —— — —— — ——
   Fluorescence with PC-1, 50-55.

D. Oleic acid ————
   Sodium oleate —— —— ——
   Fluorescence with PC-1, 55.

WAVE LENGTH, 蛣
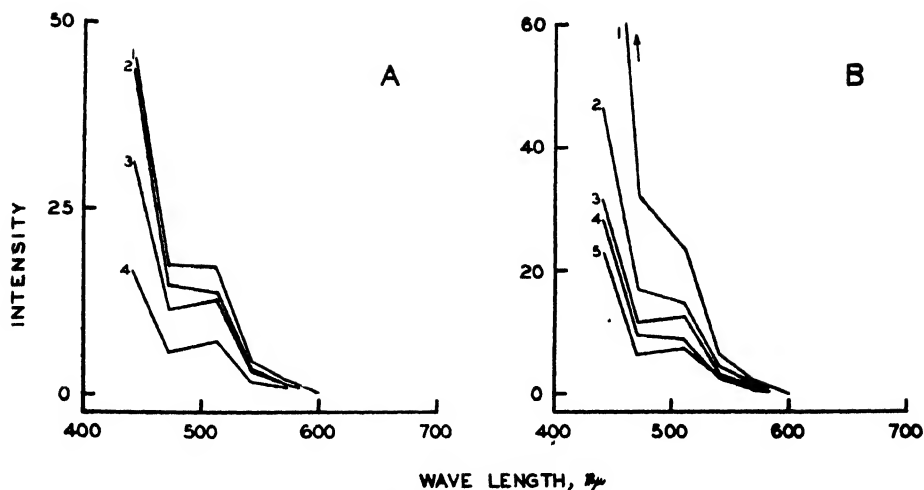
Fig. 3. **A.** Fluorescence spectra of reaction products of sugar-like
materials and amino acids in relation to β-indolyl acetic-
acid.

Diacetyl + glycine
Acetoin + glycine
1:3-Butanediol + glycine   } PC-1, 60, --Curve 1
Ethylene glycol + glycine
2:3-Butanediol + glycine; PC-1, 53, --Curve 2
Glucose + glycine
Mannose + glutamic acid } PC-1, 40, --Curve 3
β-indolyl acetic acid
Glucose + glutamic acid } PC-1, 19, --Curve 4
Acetaldehyde + glycine

B. Fluorescence spectra of protein decomposition products
in relation to B-indolyl acetic acid.
Bactotryptone; PC-1, 96, --Curve 1
Neopeptone } PC-1, 58, --Curve 2
Thiopeptone
β-indolyl acetic acid; PC-1, 40, --Curve 3
Bactopeptone; PC-1, 32, --Curve 4
Proteose peptone; PC-1, 23, --Curve 5

(All curves determined using old filters without a blank
correction).
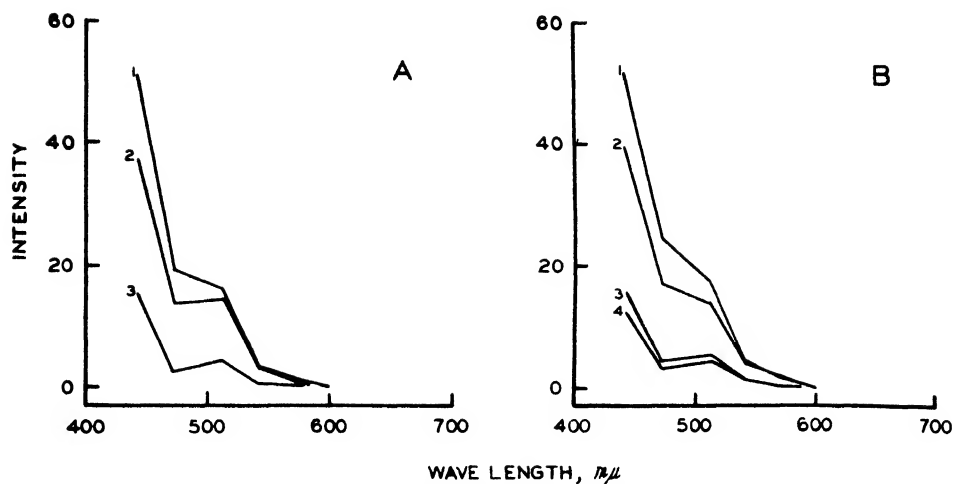
Fig. 4. A. Fluorescence spectra of potassium chloride solution
extracts of defatted egg powders of different quality.

 1. Fluorescence, 68 (identical with curve for nicotine
    at same fluorescence with filter PC-1).

 2. Fluorescence, 50.

 3. Fluorescence, 13.

 B. Fluorescence spectra of potassium chloride solution and
    ethyl alcohol (95%) extracts of defatted dried white and
    yolk.

 1. White, extracted with potassium chloride solution.

 2. Yolk, extracted with alcohol.

 3. Yolk, extracted with potassium chloride (identical
    with curve for tryptophane at same fluorescence
    value with filter PC-1).

 4. White, extracted with alcohol.

(All curves determined using old filters without a blank
 correction).

likely that this fluorescence could be attributed to furan-like materials since furfural did not fluoresce under similar conditions (Appendix I). In addition, the fluorescence spectrum of caramel differed from the spectra of egg powder extracts (cf. Fig. 7).

While many amino acids do not fluoresce (14), this examination showed that tryptophane does fluoresce and has a spectrum similar to that of pyrrole (Fig. 2). However, the limited solubility of this substance prevented any examination at high fluorescence values.

Reaction products of sugar-like materials and amino acids (reactions similar to those described in Ref. 2), and protein decomposition products all had spectra similar to materials with the pyrrole ring as a component of the molecule (Fig. 3).

Fluorescence Spectra of Egg Powder Extracts

The fluorescence spectra of egg powder extracts were similar to those of substances containing the pyrrole ring (Fig. 4). Furthermore there were at least two differentially soluble materials of this structure: one (in yolk) was more highly fluorescent in alcohol; while the other (in white) was more highly fluorescent in potassium chloride solution (Fig. 4).

It had been observed during previous work on ethanol extracts of chloroform defatted egg powder that the weight of ethanol-extractable material increased as the quality of the powder decreased (unpublished results during work described in 7). Since it was now certain that there was more than one fluorescing substance, it was decided to confine this work to the fluorescing materials in an alcohol extract of chloroform defatted egg powder.

The possibility that the fluorescing substance was tryptophane only was dismissed since this compound is so difficult to dissolve and since the greatest quantities of alcohol-soluble material were extracted from the poorest powders.

The separation procedure used is shown in Fig. 5. Cooling to -112°F. followed by warming to room temperature reduced the fluorescence value of an alcohol extract by about 25%. Although no precipitate was evident, either a portion or a specific fraction of the fluorescing material may have been rendered insoluble by this treatment. Similar cooling and warming procedures when applied to an alcohol solution of nicotine caused an increase in fluorescence (Table III), while cooling a standard solution of quinine sulphate to 45°F. did not alter its fluorescence spectra (Table II). Freezing reduced the fluorescence of a serum from fresh liquid eggs (15). These results indicated that the fluorescing substances in eggs and in egg powder were more complex than the chemicals used for reference.

The fluorescence of a solution of the hydrogen chloride precipitate shown in Fig. 5 was destroyed by neutralization. This may explain the high fluorescence values previously noted for acidified solutions of the fluorescing material (12). Since the material in the residual ether solution also fluoresced, and since an alcohol solution of these materials had a constant fluorescence at pH 6 to 10 (Fig. 5), it was now believed that the alcohol extract contained at least two fluorescing substances.

The heavy, dark brown, residual oil (Fig. 5) gave two fractions following a futile attempt to distill the material under vacuum. One of these fractions was soluble in ether and insoluble in water while the other was ether-insoluble but water-soluble. The fluorescence spectra of solutions of these two materials were similar to that of pyrrole (Fig. 6).

Absorption curves on these solutions indicated that a greater con-
centration of pyrrole was required to attain the same fluorescence
value as that of the materials causing fluorescence in egg powder
(Fig. 7). This was a further indication of the complexity of the
fluorescing materials in egg powder. A more dilute solution of pyr-
role had absorption bands between 254 and 278 mμ, and between 284 and
310 mμ. Although the absorption peaks for the water solution of the
gelatinous, ether-insoluble polymer were lower than those of pyrrole,
the bands were identical. Absorption peaks at 268 and 280 mμ and at
305 and 315 mμ were observed in other examinations of egg powder ex-
tracts (6). In the present study, the ether-soluble material had ab-
sorption peaks at 268 and 280 mμ only. All fluorescing extracts ex-
amined by absorption studies varied appreciably from the absorption at
360 mμ observed for thiochrome (16, p. 120) which has a blue fluores-
cence similar to that of pyrrole.

The water-soluble substance may be a polymer resulting from the
heat applied to the residual fluorescent oil during the attempted dis-
tillation or it may be a third fluorescing component (heat coagulable)
of the alcohol extract of defatted egg powders.

SUMMARY AND EXTENSION

The Coleman photofluorometer was modified for use as a 'fluoro-
spectrometer' by substituting for filter PC-1 nine filters which per-
mitted separation of the visible portion of the spectrum into bands
about 30 mμ in width. Using this device, pyrrole, nicotine, β-indolyl
acetic acid, and tryptophane were observed to have similar spectra.
These spectra differed slightly from those of other substances with a
blue fluorescence and differed markedly from substances with red and
green fluorescence. Protein degradation products and the reaction
products of sugar-like substances and amino acids also had fluorescence
spectra similar to pyrrole.

At least three fluorescing substances of different character and
possibly a fourth were detected in extracts of poor quality egg powder.
One, present in dried white, was highly fluorescent in potassium
chloride solution. Fluorescent material in yolk was soluble in alcohol.
Alcohol extracts of defatted whole egg powder gave a material that
fluoresced only when in acid solution, and an ether—soluble but water-
insoluble material. In addition, an ether-insoluble but water-soluble
polymer was obtained as a result of an attempt to distill the ether-
soluble fraction under reduced pressure. All fluorescing materials had
spectra almost identical with that of pyrrole and the polymer showed
absorption bands similar to pyrrole.

These results pointed to pyrrole-like substances as some of the de-
gradation products in dried egg powder and supported the evidence that
the sugar-protein reaction occurs in this product during its deterior-
ation.

While the fluorescence spectra described are crude, they were use-
ful in this work on dried egg powder. Since a similar technique has
been used to detect carcinogenic substances, a more accurate 'fluoro-
spectrometer' would be generally desirable. The proposed instrument
should be capable of providing monochromatic incident light from 200
to 600 mμ and of analyzing the emitted fluorescent light between 200
and 800 mμ. The instrument should be designed to determine spectra at
definite concentrations of fluorescing substances rather than at a
fixed maximum fluorescence, as used in the present work. It is hoped
that such an instrument, costing hundreds, rather than thousands of
dollars, can be produced in the near future either in our own labor-
atories or elsewhere.
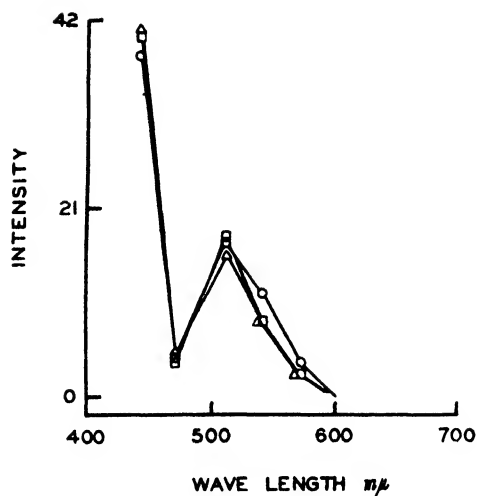
Fig. 5   Fluorescent components in egg powder

Deteriorated dried egg (high fluorescence)

Residue after defatting with chloroform

Extracted in water

Steam Dist'n

Dyalyzable but no fractionation of fluorescing material

No sep'n of fluorescing materials

Extracted in alcohol

Alcohol extract subjected to low temperature

Cooling to -112°F. reduced fluorescence by about 25%

Fractional ppt'n

Fluorescent sol'ns

Fluorescent ppts.

Same fluorescent spectra at temps. down to -35°F.

Removed alcohol by vacuum dist'n

Ether sol'n of residue

Dry hydrogen chloride added

White crystals

Sol. in alcohol and water and fluoresced with usual spectra

Neutralized with sodium hydroxide

Material not ppt'd, but now non-fluorescent

This may explain increased fluorescence in acidified egg powder extracts (12)

Residual ether sol'n fluorescent

Removed ether by vacuum dist'n

Brown fluorescent oil with pungent odour. Commenced to boil at 65°C.

Distilled under partial vacuum

Distillate: a viscous colourless, fluorescent oil with pungent odour

Fractionated at atmos. press.

Four principal fractions, all non-fluorescent.

Residue: highly fluorescent with pungent odour.

Fluorescence of alcohol solution unchanged between pH 6 and 10

Residue: heavy, dark brown, highly fluorescent oil with pungent odour

Could not be distilled at 15 mm. Hg and 100°C. Frothed badly. This heating gave

Ether-sol. fraction

Gelatinous, ether-insol polymer

See figures 6 and 7

Fig. 6 Fluorescence spectra of extracts of egg powder and pyrrole
(Flucrescence with PC-1, 52).

○   pyrrole in ether

□   ether solution of ether-soluble portion

△   water soluticn of ether-insoluble polymer

(Using new filters but without blank corrections).
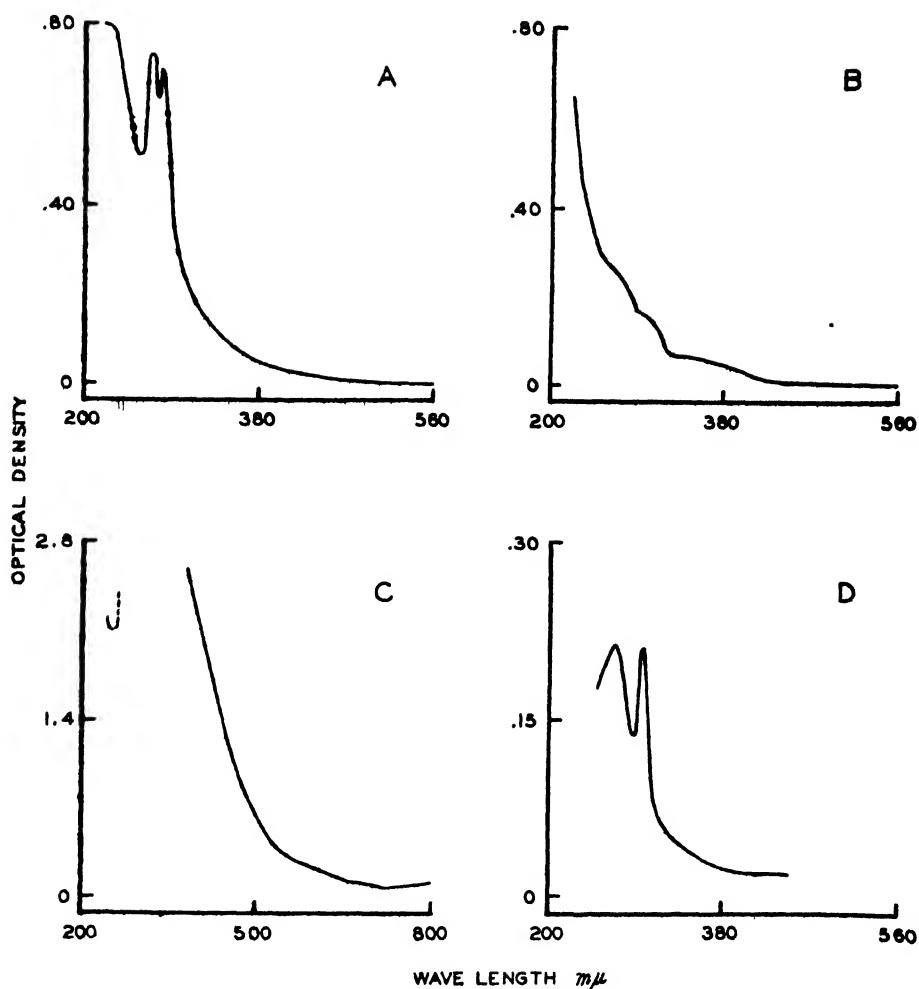
Fig. 7 Absorption spectra of extracts of egg powder and pyrrole.

    A. ether solution of ether-soluble portion

    B. water solution of ether-insoluble portion

    C. ether solution of pyrrole at same fluorescence value as
        A and B (with PC-1, 52)

    D. alcohol solution of pyrrole, more dilute than C.

ACKNOWLEDGMENTS

REFERENCES

1. Bate-Smith, E.C., and Hawthorne, J.R., J.Soc. Chem. Ind., 64: 297-302. 1945.
2. Enders, C., Kolloid-Zeit., 85: 74-87. 1938.
3. Fryd, C.F.M., and Hanson, S.W.F., J. Soc. Chem. Ind., 63: 3-6. 1944.
4. Hieger, I., Biochem. J., 24: 505-511. 1930.
5. Kipping, F.S., and Kipping, F.B., Perkin and Kipping's Organic Chemistry. J. B. Lippincott Co. Philadelphia, 1932.
6. Olcott, H.S., Dutton, H.J., and Edwards, B.G., Ind. Eng. Chem. Ind. Ed. 37: 1119-1126. 1945.
7. Pearce, J.A., Can. J. Research, D, 21: 98-107. 1943.
8. Pearce, J.A., Can. J. Research, F, 22: 87-95. 1944.
9. Pearce, J.A., Food in Canada, 5, No. 11: 24-35, 1945.
10. Pearce, J.A., Reid, M., and Cook, W.H., Can. J. Research, F, 24: 39-46. 1946.
11. Pearce, J.A., and Thistle, M.W., Can. J. Research, D, 20: 276-282. 1942.
12. Pearce, J.A., Thistle, M. W., and Reid, M., Can. J. Research, D, 21: 341-347. 1943.
13. Radley, J. A., and Grant, J., Fluorescence Analysis in Ultraviolet Light, Chapman and Hall Ltd. London, 1939.
14. Reeder, W., and Nelson, V.E., Proc. Soc. Exp. Bio. Med., 45: 792-794. 1940.
15. Reid, M., Can. J. Research, F, 24: 130-135. 1946.
16. Rosenberg, H.R., Chemistry and Physiology of the Vitamins. Interscience Publishers Inc., New York, 1942.
17. Stewart, G.F., Best, L.R., and Lowe, B., Proc. Inst. Food Tech. 1943: 77-89.
18. White, W.H., and Thistle, M.W., Can. J. Research, D, 21: 194-202. 1943.
19. Woodcock, A.H., Can. J. Research, D, 21: 90-97. 1943.

APPENDIX I

Non-fluorescing substances and substances with negligible fluorescence (filters B_1 and PC-1).

Non-fluorescing	Negligible fluorescence
o-p-hydroxybenzeneazo-p-nitrobenzene	butadiene
colchicine	thiophene
4,5-dimethyl-2-mercapto-thiazole	chlorophyll
2-methyl-1,4-napthoquinone	arginine
pyridine	proline
nicotinic acid	
ethylene diamine	
creatine	
creatinine	
sodium barbitone	
2-thion-5,5-dimethyl-oxazolidine	
4-phenyl-2-thion-thiazoline	
1,4-thiazane-4-acetic acid	
2-acetamino-thiazole	
antipyrene	
maleic anhydride	
furfural	
glycine	
glutamic acid	
methionine	
valine	
phenylalanine	
histidine	
threonine	
lysine	
leucine	

REDUCTION OF SPATIAL TEMPERATURE VARIATIONS IN AIR-COOLED STORAGE ROOMS. II.

BY J W HOPKINS, T A STEEVES, AND W. H. COOK

REDUCTION OF SPATIAL TEMPERATURE VARIATIONS IN AIR-COOLED STORAGE ROOMS. II.[1]

By J. W. Hopkins[2], T. A. Steeves[3], and W. H. Cook[4]

Abstract

Measurements made under a series of imposed conditions of heat load, dunnage spacing, and air flow were in agreement with previous work in this laboratory in demonstrating (a) the occurrence of appreciable permanent temperature gradients in material, whether exothermic or not, stacked in an air-cooled storage room, and (b) the possibility of markedly reducing these gradients by effective channelling of air through the stack. With end-to-end circulation, blocking of voids in the room was the most important single factor in minimizing intra-stack temperature differentials under the conditions of these tests. Further improvement was effected by the provision of optimum dunnage and by augmenting the air flow. It is to be inferred that with blocked voids, dunnage should be extended to all external surfaces of the stack. The desirability of uniform transverse and vertical distribution of the circulating air was also evident. Further trials on a larger scale are required to explore the practical implications of these findings.

Introduction

A previous communication from these laboratories (4) described measurements of spatial temperature variations in an experimental storage room provided with bottom-to-top air circulation. Tests were made first in the empty room, then after introducing a false load of empty boxes to obstruct the air flow, and finally after the addition of known stack heat loads generated by electrical elements placed centrally in the first layer of boxes. Presence of the boxes, even without heat, was found to increase significantly the spatial variation in temperature. Addition of heat, as expected, accentuated this effect. It was found that blocking all voids surrounding the stack itself led to a significant reduction in the spatial temperature differences within the stack. Even more pronounced reductions (of the order of 60%) resulted from the provision of dunnage spacing between each horizontal layer and also between each pair of vertical piles of boxes.

It is now desired to report upon a further and more extensive series of observations made in a somewhat larger experimental storage room. In these trials the preceding bottom-to-top air flow was replaced by an end-to-end circulation more characteristic of large warehouses, and the experiments comprised various stack heat loads, dunnage spacings, and air flows. The results demonstrate that, under the conditions specified, blocking of the voids above and at the side was by far the most important single factor in reducing

---

the accumulation of heat within the stack.   Further reductions resulted from dunnage spacing (which was optimum in the vicinity of ⅜ to ½ in.) and from increased air flow.   It is also shown that a major part of the spatial temperature differences recorded within the stack took the form of systematic gradients, transverse and vertical as well as longitudinal, and some of the features of these characteristic of various operating conditions are investigated analytically by a series of plane sections of isothermal surfaces.

Experimental

The experimental room, which was of about 1600 cu. ft. capacity, measured internally 16 ft. in length, 13 ft. in width, and 8 ft. in height.   It was insulated by a 4 in. layer of cork.   Wooden boxes of size 1 by 1 by 2 ft. were stacked in the room 12 long, 5 wide, and 6 high, giving a stack of dimensions 12 by 10 by 6 ft. exclusive of dunnage.   Dunnage (spacing strips) was applied in both the horizontal and vertical plane to each vertical group of six boxes individually, and was also applied externally to the top, bottom, and sides of the stack.   A free space of rather more than 3 ft. at the front end of the stack accommodated an exhaust blower refrigerator coil centrally located and discharging through a sheet metal duct past one side of the stack into a smaller free space, rather less than 1 ft. across, between the back of the stack and the rear wall of the room.   One side of the stack was positioned 1½ in. plus dunnage from the side wall of the room.   Insulating board placed 1 in. plus dunnage from the opposite side of the stack created a void roughly 3 ft. across between this and the opposite wall.   Through this void, which was obstructed by baffles at the front and rear (but not at the top) of the stack in order to prevent "short circuiting" of the air circulation, passed the above-mentioned air duct. The combined effect of these features was to leave free spaces of approximately 3 ft. at the front, 2 ft. at the top, 1 ft. at the back, and 1½ to 1 in. plus dunnage at the two sides of the stack.   A spacing similar to the last, namely, 1 in. plus dunnage, was also maintained between the bottom of the stack and the floor.   All boxes in the stack were equipped with electrical heating elements connected in five parallel series along the length of the stack and so installed as to ensure the generation of equal amounts of heat in all boxes at all dunnage spacings.   Thermocouples were installed at each of 23 positions in the stack as specified in Table I, and also in the exhaust and discharge air ducts.   The complete experimental arrangement is shown schematically in Fig. 1.

Two series of observations were made.   For the first the system was operated at stack heat loads of 0, 432, and 864 B.t.u. per hr. in conjunction with air flows of 500, 710, and 920 c.f.m., dunnage widths of ⅛, ⅜, and ¾ in., and with the voids above and at the side of the stack both open and blocked. For the second the voids above and beside the stack were blocked throughout but air flows of 290, 500, 710, 920, and 1130 c.f.m. were employed in conjunction with stack heat loads of 0, 432, and 864 B.t.u. per hr. as before, and with dunnage widths of 0, ¼, and ½ in.   The variable stack heat loads specified were of course additional to the more or less constant amounts entering from

without through the walls and generated inside the room by the fan motor. Calculations based on recorded differences between inlet and exhaust air temperatures indicated that these latter, which may perhaps be termed the

TABLE I

CO-ORDINATES OF LOCATIONS OF THERMOCOUPLES IN STACK, RELATIVE TO POINT SHOWN AS BOTTOM LEFT-HAND CORNER IN FIG. 1 (PLAN)

Thermocouple No.	Co-ordinates, ft.		
	Longitudinal	Transverse	Vertical
1	1 0	1 0	5 0
2	1 0	9 0	5 0
3	1 0	5 0	3 0
4	1 0	1 0	1 0
5	1 0	9 0	1 0
6	3 0	5 0	4 5
7	3 0	2 5	3 0
8	3 0	7 5	3 0
9	3 0	5 0	1 5
10	6 0	1 0	5 0
11	6 0	9 0	5 0
12	6 0	5 0	3 0
13	6 0	1 0	1 0
14	6 0	9 0	1 0
15	9 0	5 0	4 5
16	9 0	2 5	3 0
17	9 0	7 5	3 0
18	9 0	5 0	1 5
19	11 0	1 0	5 0
20	11 0	9 0	5 0
21	11 0	5 0	3 0
22	11 0	1 0	1 0
23	11 0	9 0	1 0

"maintenance heat load," averaged about 1600 B t u per hr with the voids open and about 1900 B t u per hr with the voids blocked, the difference of 300 B t u per hr. presumably representing the extra mechanical work required to maintain the same air circulation against increased resistance. The maximum stack heat load was thus of the order of 50% of the maintenance load

Blocking of the voids was effected as in the earlier experiments (4) by the inflation of suitably sized latex-coated shelter duck bags. Insulating board was interposed to maintain a space of 1 in. plus dunnage between these and the top of the stack. Ideally therefore air should have flowed with equal freedom over the top, bottom, and internal side surfaces. Actually, circulation was to some extent impeded above the stack by heater connections, and below it by thermocouple leads. With the voids blocked, therefore, the four surfaces in question were classified in the following order of increasing impedance to air flow: (i) outer side (adjacent to wall); (ii) inner side (adjacent to insulating board); (iii) top; (iv) bottom.
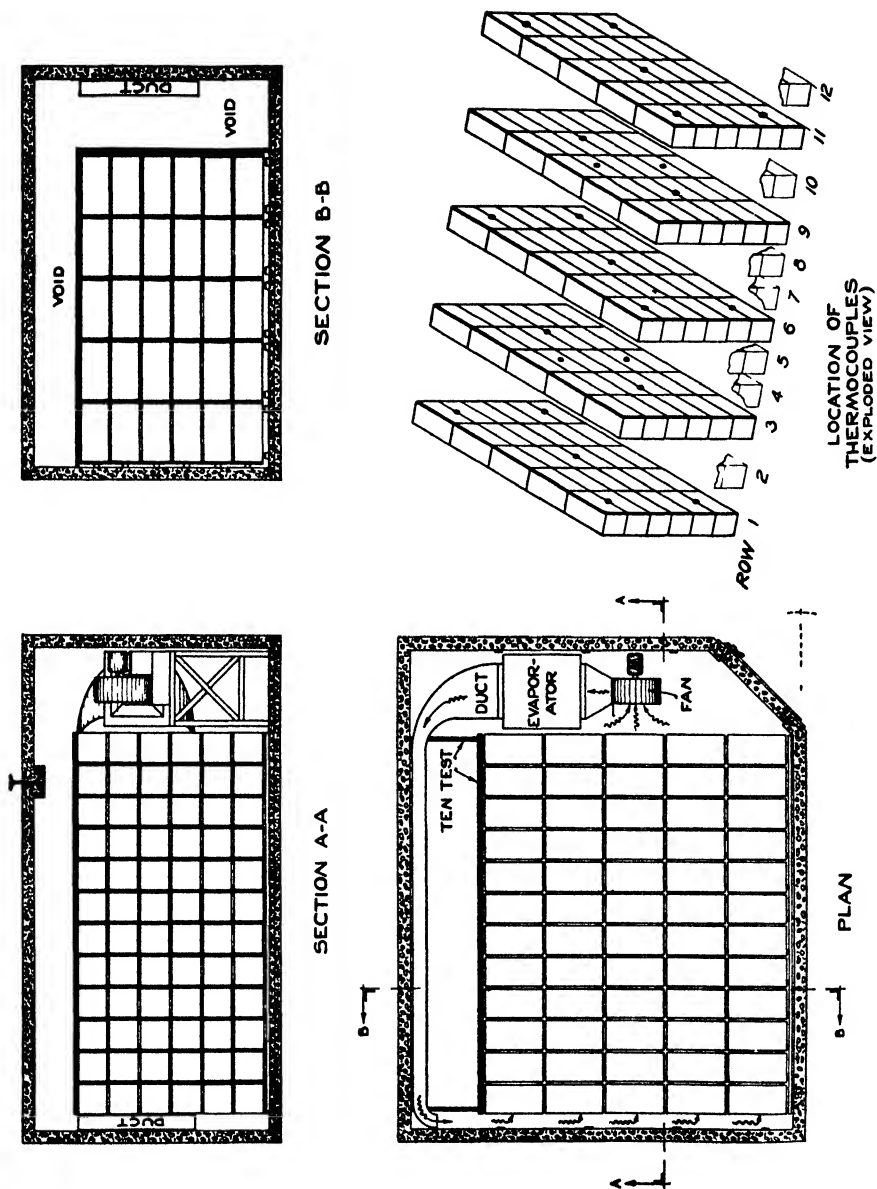
FIG 1 *Schematic diagram of test room and stack of boxes.*

After the imposition of any selected set of conditions the system was operated for several hours (usually overnight) until equilibrium was attained. Ten readings of each thermocouple were then made at 20-min. intervals and averaged to yield a mean value characteristic of the position in question. Analysis of the entire series of observations thus involved the statistical reduction of some 23,000 individual items.

Results

Assessment of Significance

Although every effort was made to operate under controlled conditions of temperature, air flow, and heat load, for a variety of reasons this aim proved to be impossible of complete achievement, and the precise temperature gradients within the stack characteristic of any particular set of imposed conditions were never completely reproducible. The residual uncontrolled variation although generally small and of random incidence was statistically significant, and in these circumstances it was necessary to have recourse to the theory of errors for assessment of the data The method adopted was to subject any function of the observations selected for study to an analysis of variance procedure (1, 2, 3) in which second-order interactions and differential effects, including the above-mentioned uncontrolled residual fluctuations, were used to test the statistical significance of differences in the averages of the main factors or their first-order interactions. The findings may conveniently be considered under the three headings of (*a*) total spatial temperature variance within the stack, (*b*) the proportion of this occurring in the form of systematic temperature gradients, and (*c*) the characteristics of such gradients.

Total Spatial Temperature Variation

The standard, i e , root-mean-square, deviation of the average reading of each of the 23 thermocouples located within the stack was used as a measure of over-all spatial variation in temperature This provides a satisfactory index of the relative variability encountered under different conditions imposed. It requires to be noted, however, that as a major portion of the variation in question proved to be systematic, the absolute values obtained in this way are applicable only to the particular ordered arrangement of the thermocouples adopted in these tests.

The main trends observable are summarized in Tables II to IV and illustrated in Fig. 2, *A–D*. Table II and Fig. 2, *A–B*, indicate that blocking the upper and side voids, thus forcing more of the circulated air to pass through the stack, markedly reduced the spatial variation at all three stack heat loads, including zero. It is to be noted, however, that whereas with the voids blocked minimum variation was recorded at $\frac{5}{8}$ in. dunnage, with the voids open the widest spacing used ($\frac{3}{4}$ in.) gave the least variation, suggesting that in this circumstance still wider spacing might have been advantageous. Table III and Fig. 2, *C*, obtained by combining relevant portions of both the first and second series of experiments, permit a comparison of six dunnage

TABLE II

INDEX OF SPATIAL TEMPERATURE VARIATION (STANDARD DEVIATION IN DEG. F.) FOR VARIOUS CONDITIONS OF DUNNAGE, AIR FLOW, AND HEAT LOAD

Dunnage, in.	Air flow, c.f.m.	Voids open				Voids blocked			
		Stack heat load				Stack heat load			
		0	432 B.t.u./hr.	864 B.t.u./hr.	Average	0	432 B.t.u./hr.	864 B.t.u./hr.	Average
⅛	500	2.3	2.9	3.7	2.95	0.7	1.5	2.2	1.47
	710	2.4	2.9	3.2	2.80	0.9	1.8	2.5	1.76
	920	2.0	3.1	3.6	2.89	0.4	1.8	2.4	1.52
	Average	2.23	2.93	3.44	2.87	0.67	1.71	2.36	1.59
⅜	500	2.6	3.1	3.8	3.14	0.8	1.2	1.2	1.08
	710	2.8	3.0	3.2	3.01	0.6	0.8	1.1	0.83
	920	2.6	2.6	3.2	2.78	0.5	0.7	0.9	0.71
	Average	2.64	2.90	3.39	2.98	0.64	0.91	1.07	0.87
¾	500	2.3	2.7	2.8	2.59	1.2	1.5	1.8	1.17
	710	1.9	2.4	2.7	2.33	0.8	1.3	1.5	1.22
	920	2.0	2.3	2.5	2.27	0.9	1.2	1.2	1.11
	Average	2.07	2.45	2.67	2.40	0.98	1.33	1.52	1.28
Average		2.32	2.76	3.18	2.75	0.77	1.32	1.65	1.24

Necessary difference (5% level of statistical significance) between averages of 3 = ± 0.30.
Necessary difference (5% level of statistical significance) between averages of 9 = ± 0.17.
Necessary difference (5% level of statistical significance) between averages of 27 = ± 0.11.

TABLE III

INDEX OF SPATIAL TEMPERATURE VARIATION (STANDARD DEVIATION IN DEG. F.) UNDER SPECIFIED CONDITIONS OF DUNNAGE AND HEAT LOAD.

(AVERAGES FOR AIR FLOWS OF 500, 710, AND 920 C.F.M. VOIDS BLOCKED)

Stack heat load, B.t.u./hr.	Dunnage spacing, in.					
	0	⅛	¼	⅜	½	¾
0	0.75	0.68	0.74	0.64	0.67	0.98
432	1.90	1.71	1.47	0.91	0.83	1.33
864	3.15	2.36	1.97	1.07	1.02	1.52

Necessary difference for 5% level of statistical significance = ± 0.28.

spacings ranging from 0 to ¾ in., with the voids blocked. Under these conditions, optimum dunnage was clearly in the neighbourhood of ⅜ to ½ in., whilst the effect of reducing this spacing became progressively more pronounced with increasing heat load. Table IV and Fig. 2, D, constructed entirely from the second series of trials, show that increasing the air flow

TABLE IV

INDEX OF SPATIAL TEMPERATURE VARIATION (STANDARD DEVIATION IN DEG. F.) UNDER SPECIFIED CONDITIONS OF AIR FLOW AND HEAT LOAD.

(AVERAGES FOR 0, $\frac{1}{2}$, AND $\frac{1}{4}$ IN. DUNNAGE.   VOIDS BLOCKED)

Air flow, c f.m.	Stack heat load, B.t.u./hr.		
	0	432	864
290	1 25	2 12	2.94
500	0 92	1 58	2 41
710	0 65	1 32	1 90
920	0 60	1 31	1 84
1130	0 46	1 19	1 70

Necessary difference for 5% level of statistical significance = ± 0 23.
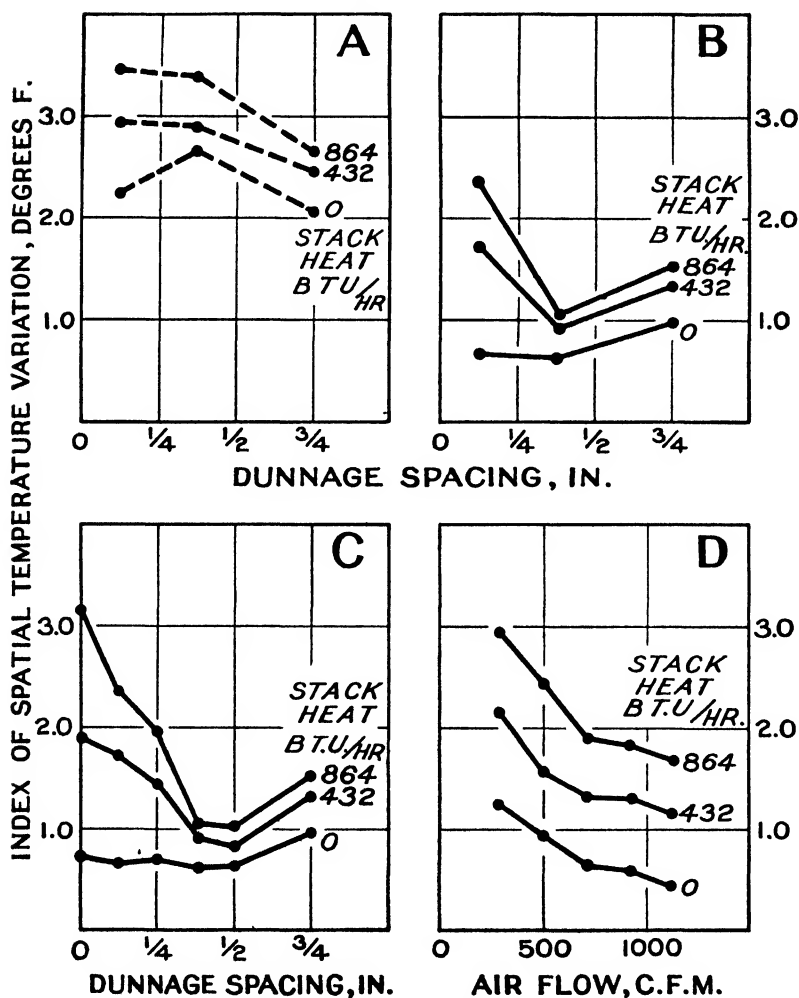
FIG. 2. *Index of spatial temperature variation (standard deviation in deg. F.) in relation to dunnage, heat load, and air flow.   A: voids open.   B–D: voids blocked.*

from 290 to 1130 c.f.m., i.e., from about 11 to about 40 changes per hour in the empty room, led to a progressive, although not directly proportional reduction in temperature variations. This was true of all the three heat loads and dunnage spacings included in this series.

Temperature Gradients

Systematic elements in the foregoing temperature differences within the stack were investigated by equating the mean temperature t characteristic of each of the 23 measured positions to a general quadratic function of the rectangular co-ordinates x, y, z of the point in question. This equation was of the form:

$$t = a + bx + cy + dz + exy + fxz + gyz + hx^2 + iy^2 + jz^2 + \epsilon \quad (1)$$

Values of the 10 coefficients a–j were determined by the method of Least Squares so as to minimize in turn the sum of the squares of the residuals $S(\epsilon^2)$ for each of the sets of operating conditions included in the experiments. The difference between $S(\epsilon^2)$ and $S(t-\bar{t})^2$, where $\bar{t}$ denotes the average of the 23 recorded mean temperatures, then provided a measure of the proportion of the total intra-stack temperature variance representable by continuous quadratic gradients. This is set forth in the form of percentages in Tables V to VII.

TABLE V

PERCENTAGE OF INTRA-STACK TEMPERATURE VARIANCE
REPRESENTABLE BY QUADRATIC GRADIENTS.
(AVERAGES FOR $\frac{1}{8}$, $\frac{3}{8}$, AND $\frac{3}{4}$ IN
DUNNAGE AND AIR FLOWS OF 500,
710, AND 920 C Γ M.)

Stack heat load, B t u /hr	Voids	
	Open	Blocked
0	88	90
432	88	92
864	86	91
Average	87	91

Augmented air flow (Table VI) or heat load in conjunction with $\frac{1}{4}$ in. or less dunnage (Table VII) resulted in some increase in the complexity of the heat distribution, which was accordingly less adequately represented by a quadric surface. In general nevertheless the quadratic function specified in Equation (1) accounted for some 90% of the total intra-stack variance, indicating that by far the greater part of the recorded temperature differences were associated with relatively simple continuous gradients, however different the form of these might be under the various conditions of heat load, dunnage, and air flow. It may be remarked that the terms in xy, xz, and yz in Equation (1) are to be interpreted physically as representing interactions of the linear temperature gradients in the x, y, and z directions. Such interactions would

TABLE VI

PERCENTAGE OF INTRA-STACK TEMPERATURE VARIANCE
REPRESENTABLE BY QUADRATIC GRADIENTS.

(AVERAGES FOR 0, $\frac{1}{4}$, AND $\frac{3}{4}$ IN. DUNNAGE. VOIDS BLOCKED)

Air flow, c.f.m.	Stack heat load, B.t.u./hr.		
	0	432	864
290	94	90	93
500	93	90	89
710	91	90	88
920	91	89	90
1130	81	89	87

TABLE VII

PERCENTAGE OF INTRA-STACK TEMPERATURE VARIANCE
REPRESENTABLE BY QUADRATIC GRADIENTS.

(AVERAGES FOR AIR FLOWS OF 500, 710, AND 920 C F.M   VOIDS BLOCKED)

Stack heat load, B.t.u./hr.	Dunnage spacing, in.					
	0	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{3}{8}$	$\frac{1}{2}$	$\frac{3}{4}$
0	92	92	96	87	86	90
432	89	88	91	93	91	94
864	89	86	85	94	93	94

operate to produce for example a modification of the longitudinal gradient as between the bottom and top or one side and the other of the stack.   The occurrence of several such interactions was revealed by the analyses of variance.   Their numerical magnitude was however in all instances definitely secondary to that of the average linear and quadratic trends.

Characteristics of Temperature Gradients

Tables VIII to X summarize the mean linear gradients deduced from the temperature differences recorded within the stack under various conditions. The mean longitudinal gradients listed range from + 0 64° F. per ft. for a stack heat load of 864 B.t.u. per hr. with the voids open (Table VIII) to + 0 07° F. per ft. for zero stack heat and 1130 c.f.m. air flow with the voids blocked (Table IX).   In general the longitudinal gradient was reduced by more than 50% by blocking the voids or by increasing the air flow from 290 to 1130 c.f.m.   It was rather less sensitive to variations in dunnage (Table X) but was at a minimum in the region of $\frac{3}{8}$ to $\frac{1}{2}$ in.   The mean linear transverse and vertical gradients were for the most part small in magnitude and fluctuated irregularly in sign, indicating that the temperature differences recorded in these directions were distributed fairly symmetrically about the centre of the

TABLE VIII

MEAN LINEAR TEMPERATURE GRADIENTS (DEG. F. PER FT.). AVERAGES FOR ⅛, ⅜, AND ⅝ IN. DUNNAGE AND AIR FLOWS OF 500, 710, AND 920 C.F.M.

Stack heat load, B.t.u./hr.	Longitudinal Voids		Transverse Voids		Vertical Voids	
	Open	Blocked	Open	Blocked	Open	Blocked
0	+0.47	+0.13	+0.16	−0.03	−0.38	+0.01
432	+0.58	+0.24	+0.19	−0.06	−0.37	+0.04
864	+0.64	+0.34	+0.21	−0.10	+0.20	+0.06
Average	+0.56	+0.24	+0.19	−0.06	−0.13	+0.04

Necessary difference for 5% level of statistical significance: Longitudinal, ±0.04; transverse, ±0.02; vertical, ±0.04.

TABLE IX

MEAN LINEAR TEMPERATURE GRADIENTS (DEG. F. PER FT.).
AVERAGES FOR 0, ¼ AND, ½ IN. DUNNAGE. VOIDS BLOCKED

Air flow, c.f.m.	Longitudinal Stack heat load, B.t.u./hr.			Transverse Stack heat load, B.t.u./hr.			Vertical Stack heat load, B.t.u./hr.		
	0	432	864	0	432	864	0	432	864
290	+0.27	+0 41	+0 54	−0 02	+0 02	+0 03	−0.11	−0 09	+0.27
500	+0.16	+0 29	+0 41	−0 02	−0 06	−0 05	−0 12	−0 03	+0.05
710	+0.18	+0.27	+0.34	+0 01	−0 04	−0 07	−0 07	+0 01	+0.01
920	+0 11	+0 22	+0 29	+0 00	+0 01	+0 02	+0 01	+0 02	+0.04
1130	+0.07	+0.21	+0 24	+0 00	+0 02	+0 07	+0 01	−0 01	−0.00

Necessary difference for 5% level of statistical significance; longitudinal, ± 0 07; transverse, ± 0.05; vertical, ± 0 14.

TABLE X

MEAN LINEAR TEMPERATURE GRADIENTS (DEG. F. PER FT.)
AVERAGES FOR AIR FLOWS OF 500, 710, AND 920 C.F.M. VOIDS BLOCKED

Dunnage, in.	Longitudinal Stack heat load, B t u /hr			Transverse Stack heat load, B.t.u./hr.			Vertical Stack heat load, B.t.u./hr.		
	0	432	864	0	432	864	0	432	864
0	+0 15	+0 29	+0.42	−0.10	−0 12	−0.14	−0.08	−0.08	−0.02
⅛	+0.09	+0.25	+0 44	−0.10	−0.23	−0.36	+0.09	+0.10	+0 11
	+0 20	+0 32	+0.40	+0.05	−0.04	−0.04	−0 07	−0.05	−0.08
	+0.12	+0 21	+0.26	−0.01	−0.00	−0.01	+0.16	+0 06	+0.05
	+0.10	+0.17	+0.23	+0.05	+0.07	+0.08	+0.06	+0 08	+0.17
	+0.19	+0 26	+0.33	+0.03	+0 06	+0 08	−0.13	−0 08	−0.04

Necessary difference for 5% level of statistical significance: Longitudinal, ± 0.06; transverse, ± 0.06; vertical, ± 0.10.

stack. They, however, present no information respecting the pronounced curvature actually characteristic of these gradients. This was examined analytically as described below.

If t is given some constant value such as $t_0 + 1$, where t_0 is the mean temperature of the cold air entering the room from the delivery duct, and if ϵ is neglected, then Equation (1) specifies the quadric surface approximating most closely to the actual surface generated by the aggregate of points within the stack at the temperature $t_0 + 1$. This may be termed the isothermal surface of $+ 1°$. Similarly equating t to $t_0 + 2$, $t_0 + 3$, $---- t_0 + n$ leads to the isothermal surfaces of $+ 2°$, $+ 3°$, $---- + n°$. Figs 3 to 7 illustrate some of the characteristics of these surfaces under different experimental conditions by longitudinal, transverse, and vertical plane sections through the central axes of the stack.
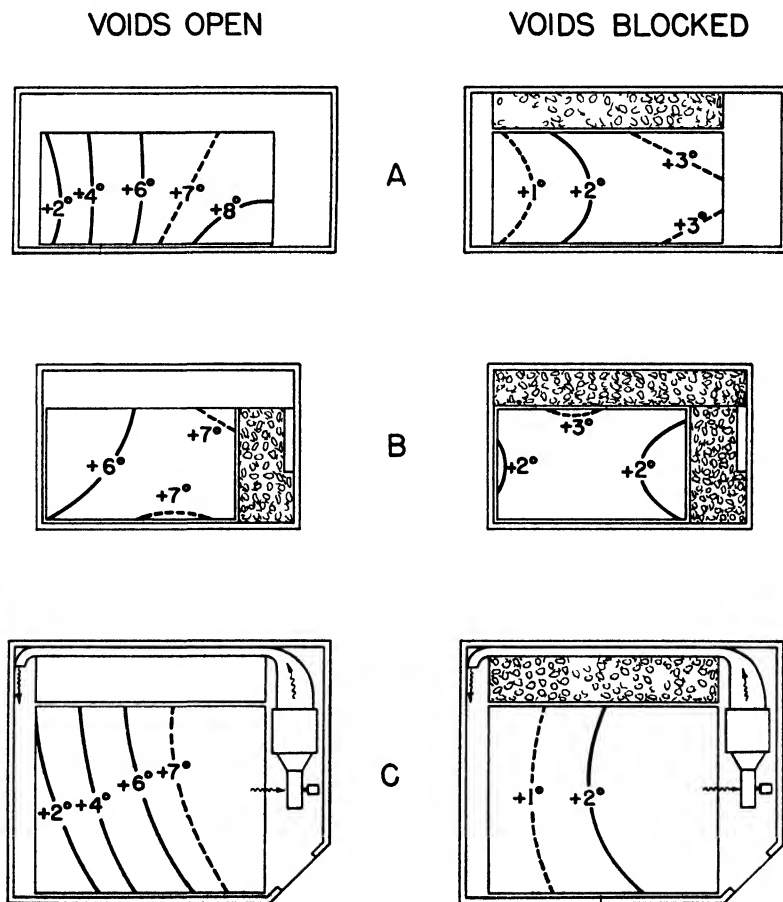

FIG 3 *Plane sections of isothermal surfaces. A: central longitudinal vertical section. B: central transverse vertical section.   C: central longitudinal horizontal section.   Averages for air flows of 500, 710, 920 c.f m , heat loads of 0, 432, and 864 B t u. per hr., and ½, ⅜, and ¼ in. dunnage.*

A considerable number of the surfaces obtained were sectors of hyperboloids of one sheet. They thus exhibited anticlastic ("saddle-shaped") curvature, presumably owing to the fact that cold air circulated across the sides of the stack more freely than across the top or bottom. However, the general breaking down of temperature gradients by an air flow of 1130 c.f.m. with the voids blocked resulted in an "open" isothermal surface for $+ 2°$ F. which was approximated by the portion of a hyperboloid of two sheets illustrated in Fig. 6. Various segments of ellipsoids also resulted.

Fig. 3 portrays the marked decrease in both magnitude and complexity of temperature gradients resulting from blocking the voids and thereby channelling circulating air more effectively through the stack. Also to be noted is the asymmetry of the temperature gradients obtained with the void open. It would appear that in this circumstance, the non-central location of the discharge duct tended towards an orientation of the air circulation across and over the surface of the stack. This characteristic was largely, although not completely, eliminated by blocking the voids, the previously noted differential impedance of air movement across the top, bottom, and sides of the stack still remaining operative. The foregoing effects are observable in more detail in Figs. 4 and 5. These also illustrate the building up of temperature
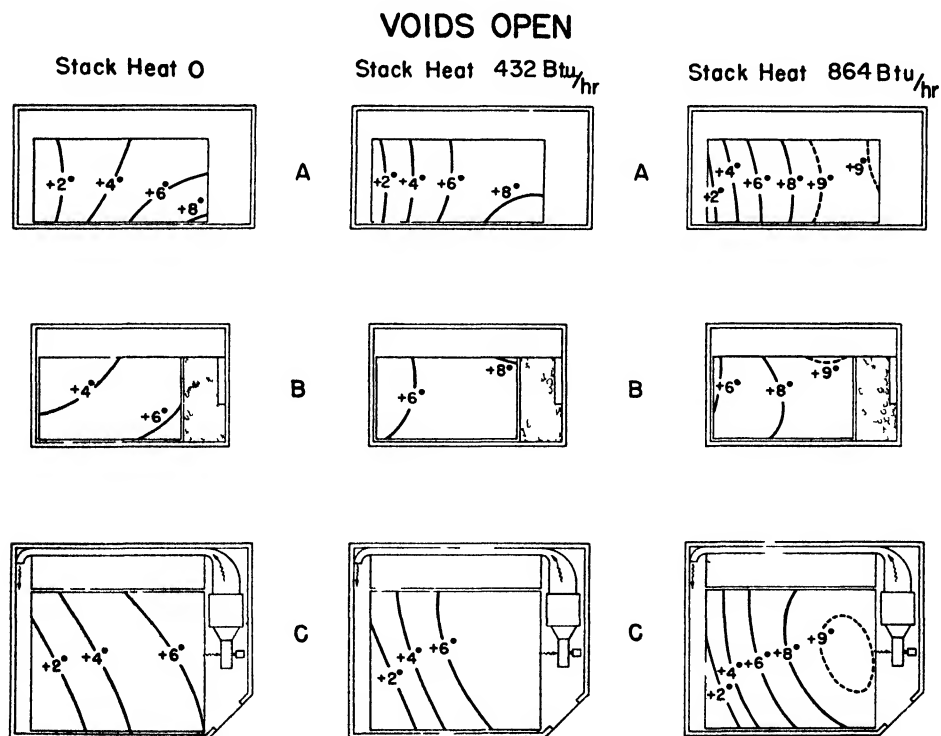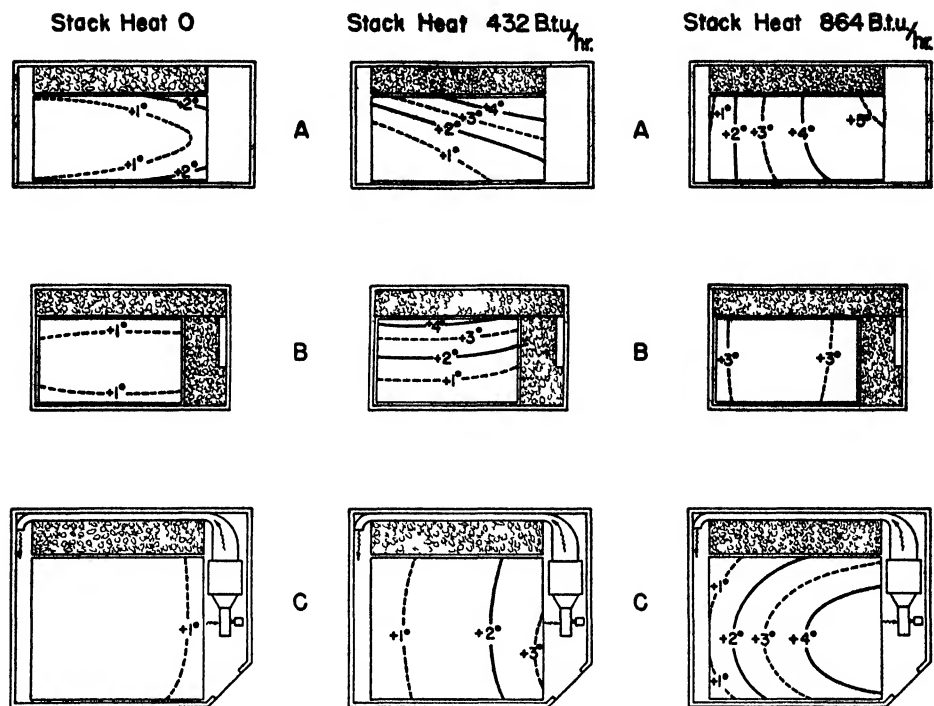
VOIDS OPEN

FIG. 4. *Plane sections of isothermal surfaces, voids open. A: central longitudinal vertical section B: central transverse vertical section. C: central longitudinal horizontal section. Averages for air flows of 500, 710, and 920 c.f.m. and ⅛, ⅜, and ¾ in. dunnage.*

stack. They, however, present no information respecting the pronounced curvature actually characteristic of these gradients. This was examined analytically as described below.

If t is given some constant value such as $t_0 + 1$, where t_0 is the mean temperature of the cold air entering the room from the delivery duct, and if ϵ is neglected, then Equation (1) specifies the quadric surface approximating most closely to the actual surface generated by the aggregate of points within the stack at the temperature $t_0 + 1$. This may be termed the isothermal surface of $+ 1°$. Similarly equating t to $t_0 + 2$, $t_0 + 3$, $---- t_0 + n$ leads to the isothermal surfaces of $+ 2°$, $+ 3°$, $---- + n°$. Figs. 3 to 7 illustrate some of the characteristics of these surfaces under different experimental conditions by longitudinal, transverse, and vertical plane sections through the central axes of the stack.

VOIDS OPEN   VOIDS BLOCKED

A

B

C

Fig 3 *Plane sections of isothermal surfaces. A: central longitudinal vertical section.*
B: central transverse vertical section.   C· central longitudinal horizontal section. Averages for
air flows of 500, 710, 920 c.f m., heat loads of 0, 432, and 864 B t.u. per hr , and ⅛, ⅜, and
⅝ in. dunnage.

A considerable number of the surfaces obtained were sectors of hyperboloids of one sheet. They thus exhibited anticlastic ("saddle-shaped") curvature, presumably owing to the fact that cold air circulated across the sides of the stack more freely than across the top or bottom. However, the general breaking down of temperature gradients by an air flow of 1130 c.f.m. with the voids blocked resulted in an "open" isothermal surface for $+ 2°$ F. which was approximated by the portion of a hyperboloid of two sheets illustrated in Fig. 6. Various segments of ellipsoids also resulted.

Fig. 3 portrays the marked decrease in both magnitude and complexity of temperature gradients resulting from blocking the voids and thereby channelling circulating air more effectively through the stack. Also to be noted is the asymmetry of the temperature gradients obtained with the void open. It would appear that in this circumstance, the non-central location of the discharge duct tended towards an orientation of the air circulation across and over the surface of the stack. This characteristic was largely, although not completely, eliminated by blocking the voids, the previously noted differential impedance of air movement across the top, bottom, and sides of the stack still remaining operative. The foregoing effects are observable in more detail in Figs. 4 and 5. These also illustrate the building up of temperature
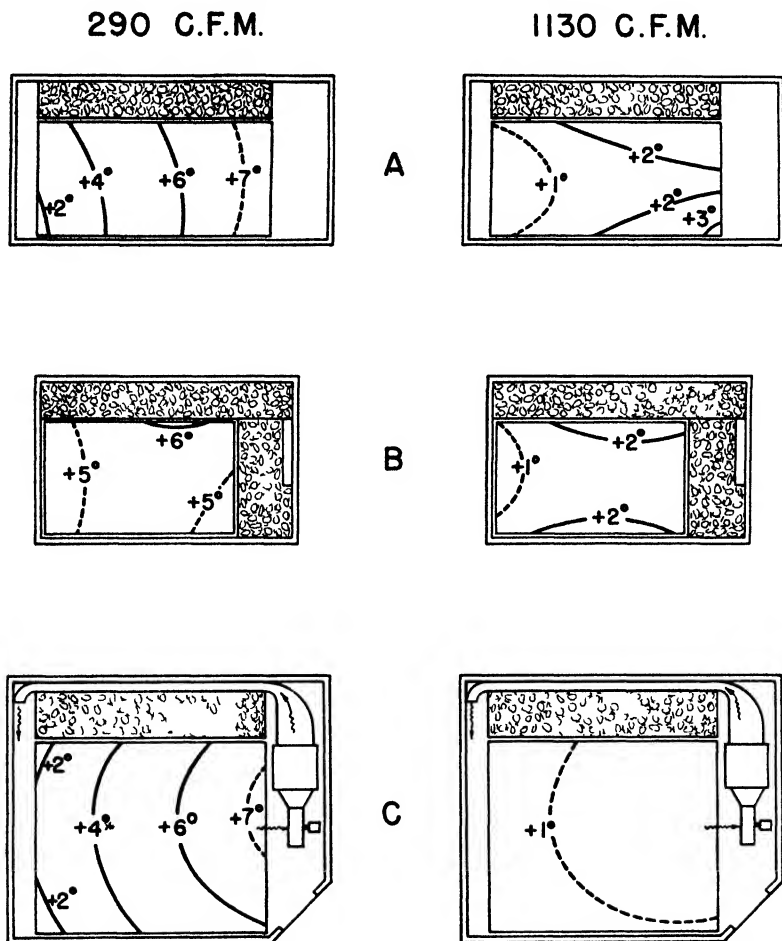
VOIDS OPEN

FIG. 4. *Plane sections of isothermal surfaces, voids open. A: central longitudinal vertical section B: central transverse vertical section. C: central longitudinal horizontal section. Averages for air flows of 500, 710, and 920 c.f.m. and ⅛, ¼, and ¾ in. dunnage.*
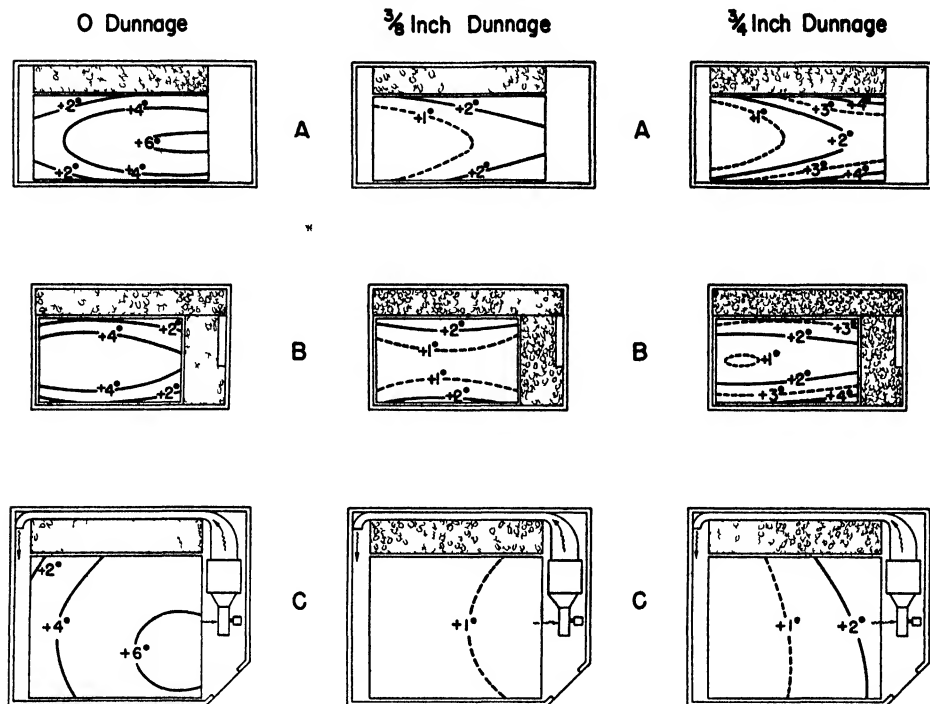
VOIDS BLOCKED

FIG 5. *Plane sections of isothermal surfaces, voids blocked. A: central longitudinal vertical section. B· central transverse vertical section. C: central longitudinal horizontal section. Averages for air flows of 500, 710, and 920 c f m. and ⅜, ½, and ¾ in. dunnage.*

gradients by stack heat loads, whilst Fig. 6 exemplifies their reduction, particularly in the central zone of the stack, by augmented air flow. Fig. 7 shows the average gradients recorded with the voids blocked for zero, ⅜ and ¾ in. dunnage, the second of these being in the vicinity of the optimum for the operating conditions of these tests.

This optimum was presumably occasioned by the balancing of two opposed factors as follows. Maintenance of an extra inch or 1½ in. of free space in addition to dunnage at the top, bottom, and sides of the stack must have resulted in a correspondingly greater part of the total circulated air passing over these surfaces rather than through the apertures in the stack itself. This in turn would lead to relatively more accumulation of heat in the centre which, in the event of the stack apertures being small, would more than offset the effect on the 'external surfaces of radiation from the walls, ceiling, and floor. The left-hand side of Fig. 7 illustrates this effect in the extreme case of zero dunnage, i.e., no internal aperture at all. As the size of the internal apertures was increased, more air traversed the stack internally, and ⅜ in. dunnage sufficed to reduce temperatures at the centre below those at the periphery, as shown in the middle portion of Fig. 7. On the other hand,

increasing the width of the apertures by additional dunnage must have correspondingly reduced the linear velocity associated with the circulation of a specified number of cubic feet of air per minute, until eventually a point was reached at which this became a limiting factor, and a further accumulation of

VOIDS BLOCKED

290 C.F.M.   1130 C.F.M.

FIG. 6. *Plane sections of isothermal surfaces, voids blocked. A: central longitudinal vertical section.   B: central transverse vertical section.   C: central longitudinal horizontal section.   Averages for heat loads of 0, 432, and 864 B.t.u. per hr.*

heat, particularly peripherally but to some extent internally as well, became manifest.   This would appear to be the situation exhibited in the right hand portion of Fig. 7.

It will be appreciated that the quadric surfaces delineated in Figs. 3 to 7 are only second-degree approximations to the actual isothermal surfaces generated within the stack.   From Tables V to VII, however, it is clear that

they must in fact portray fairly closely most of the recorded temperature gradients.   In general these gradients were characterized by more pronounced curvature in the vertical than in either the horizontal or transverse planes,

VOIDS BLOCKED

FIG. 7. *Plane sections of isothermal surfaces, voids blocked   A: central longitudinal vertical section.   B: central transverse vertical section: C: central longitudinal horizontal section   Averages for heat loads of 0, 432, and 864 B.t.u. per hr. and air flows of 500, 710, and 920 c.f.m.*

presumably as a further consequence of the unequal restriction of air movement over the top, bottom, and side surfaces.   Dunnage eliminating vertical and transverse gradients and reducing all isothermal surfaces to planes at right angles to the direction of air flow would be desirable as indicative of uniform effectiveness of heat removal throughout the stack.   An approximation to this might perhaps be achieved by using different widths of dunnage at the centre, periphery, and exterior, although the requirements would almost certainly be modified by any alteration in the size or shape of the stack or in the location of inlet and exhaust ports.

Conclusions

It is concluded that these experiments were in agreement with previous work in this laboratory in demonstrating (*a*) the occurrence of appreciable permanent temperature gradients in material, whether exothermic or not,

stacked in an air-cooled storage room, and (*b*) the possibility of markedly reducing these gradients by effective channelling of air through the stack. With end-to-end circulation, blocking of voids in the room was the most important single factor in minimizing intra-stack temperature differentials under the conditions of these tests. Further improvement was effected by the provision of optimum dunnage and by augmenting the air flow. It is to be inferred that with blocked voids, dunnage should be extended to all external surfaces of the stack. The desirability of uniform transverse and vertical distribution of the circulating air was also evident. Further trials on a larger scale are required to investigate the extent to which the present results were a function of the size and shape of the stack, and also to determine the most suitable ratio of external to internal dunnage.

Acknowledgments

References

1. BROWNLEE, K. A. Industrial experimentation. Directorate of Royal Ordnance Factories, Ministry of Supply, London. Memo. No. 18. 1945.
2. FISHER, R. A. Statistical methods for research workers. 8th ed. Oliver and Boyd, London. 1941.
3. FREEMAN, H. A. Industrial statistics. John Wiley and Sons, Inc., New York. 1942.
4. STEEVES, T. A. and COOK, W. H. Can. J. Research, F, 23 : 253-262. 1945.

PACKAGING

IV. METHODS OF APPLYING WATER-VAPOUR BARRIERS, AND THE WATER-VAPOUR RESISTANCE OF SOME PACKAGING MATERIALS[1]

By C. G. Lavers[2] and Jesse A. Pearce[3]

Abstract

Reynolds' Metal A–10 and 450 M.S.Y.T. "Cellophane" were used as liners and overwraps and Darex P–16 as a wax-dip for cartons containing sawdust, and packed in a master container. Some packages were dropped a distance of three feet, 20 times at −40° F., others received the same treatment at room temperature, and some were subjected to a free fall of about 70 ft. Greatest protection was provided by the use of a liner inside the carton.

Water-vapour resistance and ability to withstand rough handling were investigated for a wide variety of packaging materials (all materials but one tested as carton liners). Laminated materials having metal foil as one layer provided the greatest protection. Wax-coatings effectively reduced water-vapour transmission, but provided little added protection when packages were subjected to shock. Laminating two stocks produced marked reduction in the water-vapour transmission typical of either base sheet when used alone. Combinations utilizing scrim or kraft produced barriers that were less likely to fracture when subjected to rough handling. When Cellophane was considered, M.S.Y.T. stock and the use of triplex bags provided the greatest protection.

Introduction

The need for protection against loss or gain of water vapour by foods, particularly when frozen or dehydrated, is generally recognized in the food industry today. For this reason, a limited study of the water-vapour resistance of packaging materials has already been made in this laboratory (3). It was felt desirable to continue this work and evaluate thoroughly the effectiveness of the more important types of flexible barriers available in Canada.

During the course of preliminary studies (3), the question of the relative effectiveness of carton liners and overwraps was raised. In addition, no precise information was available to permit selection between the foregoing methods and wax-dipping as a means of providing protection against water-vapour penetration. Hence, prior to investigating individual materials an experiment was designed to evaluate the resistance to rough handling of packages made by these various methods.

Relative Fragility of Water-vapour Barriers when Applied by Various Methods

Materials and Methods

The packaging materials selected for use in this portion of the investigation were: 450 M.S.Y.T. "Cellophane," Reynolds' Metal A–10 (a lamination of kraft to metal foil to Cellophane), and Darex Wax P–16 (a commercial dipping wax). These materials were used to prepare liners and overwraps or for wax-dipping 12 cartons, 4 by $2\frac{3}{4}$ by $1\frac{5}{16}$ in. (opening end $2\frac{3}{4}$ by $1\frac{5}{16}$ in.). These cartons were of the regular flat folding style with full overlapping long flaps and were made of 0 020 in. chipboard. The packages were filled with sawdust, closed, and packed in a master container which accommodated three layers each containing six by four or 24 upright packages. The master container was $12\frac{1}{4}$ by $11\frac{1}{8}$ by $12\frac{1}{4}$ in. high (opening end $12\frac{1}{4}$ by $11\frac{1}{8}$ in.), and was made of regular B-flute, utilizing $0.016 - 0.009 - 0.016$ Fourdrinier kraft liner and corrugations. Of 72 cartons in each master container, a set of 12 was under study and were packed in fixed positions (Fig. 1), the remaining space being filled with dummy packages containing sawdust.

Three experimental conditions of rough handling were investigated. The first involved cooling the packages to minus 40° F., and then dropping them 20 times through a distance of three feet to a cement floor (five falls on each of the four upright edges of the master container). The second treatment followed the same procedure at room temperature (approximately 75° F.). The third permitted a single fall of about 70 ft. on to cement (temperature of packages, approximately 75° F.). After treatment the packages were opened and the water-vapour barriers were examined visually for fractures, and for pin holes.

Results

The results of the visual examination of the packaging materials are given in Table I, and the data are summarized on a per cent basis in Table II. Of the materials used, Cellophane did not develop pin holes as easily as did Reynolds' Metal A–10, but it was more prone to fracture. Over-all, Cellophane appeared slightly more flexible than Reynolds' Metal, but this was not borne out by subsequent work, as will be shown later.

Reduction in temperature greatly increased the fragility of barriers when roughly handled. In addition, a number of short falls, a condition more likely to be met in ordinary commercial handling and transport, caused greater damage to the water-vapour barrier than a single fall from a much greater height.

Under the handling conditions described, wax-dipping was generally less desirable than either of the other two methods of providing water-vapour protection. While Darex P–16 may not be the most flexible wax obtainable,

TABLE I

FRAGILITY OF WATER-VAPOUR BARRIERS WHEN ROUGHLY HANDLED

Method of providing water-vapour barrier	Method of handling	Number of barriers		
		Unbroken	With pinholes	Fractured
Cartons with liners				
450 M.S.Y.T. Cellophane ⎫ Reynolds' Metal A–10 ⎬	3 ft. drop, 20 falls at −40° F.	{ 2 { 0	2 10	8 2
450 M.S.Y.T. Cellophane ⎫ Reynolds' Metal A–10 ⎬	3 ft. drop, 20 falls at approx. 75° F.	{ 7 { 3	2 9	3 0
450 M.S.Y.T. Cellophane ⎫ Reynolds' Metal A–10 ⎬	70 ft. drop (approx.), one fall at approx. 75° F.	{11 { 9	1 1	0 2
Overwrapped cartons				
450 M.S.Y.T. Cellophane ⎫ Reynolds' Metal A–10 ⎬	3 ft. drop, 20 falls at −40° F.	{ 0 { 0	0 2	12 10
450 M.S.Y.T. Cellophane ⎫ Reynolds' Metal A–10 ⎬	3 ft. drop, 20 falls at approx 75° F.	{ 4 { 0	4 9	4 3
450 M.S.Y.T. Cellophane ⎫ Reynolds' Metal A–10 ⎬	70 ft. drop (approx), one fall at approx. 75° F.	{ 8 { 6	1 3	3 3
Wax-dipped cartons				
Darex P–16	3 ft. drop, 20 falls at −40° F.	0	0	12
Darex P–16	3 ft. drop, 20 falls at approx 75° F.	0	0	12
Darex P–16	70 ft. drop (approx), one fall at approx. 75° F.	0	0	12

the results are nevertheless indicative of what may be expected from this type of packaging using currently available waxes. It has been observed elsewhere· (2) that rough handling caused an appreciable increase in the moisture gain of wax-dipped packages of dehydrated pork.

The most desirable method of providing water-vapour piotection was a liner inside the carton (Table II). This method cannot be used, however, when the contents of the package are of such a nature as to effect possible rupture of the inner barrier. In such circumstances overwrapped cartons seemed to be more desirable. Subsequent work has shown, however, that the best method of packaging such materials is by the use of the container–barrier–container method (1). This consists of placing the product in a light carton, applying the water-vapour barrier, and placing the whole in another snugly fitting carton.

TABLE II

SUMMARY OF FACTORS AFFECTING THE FRAGILITY OF WATER-VAPOUR BARRIERS
CONSIDERED OVER OTHER CONDITIONS

Factor	Per cent of barriers		
	Unbroken	With pinholes	Fractured
Method of providing barrier			
Liner	44	35	21
Overwrap	25	26	49
Wax-dip	0	0	100
Material			
Cellophane	44	14	42
Reynolds' Metal A–10	25	47	28
Darex P–16	0	0	100
*Method of handling**			
3 ft drop, 20 falls at −40° F	4	29	67
3 ft drop, 20 falls at approx 75° F	29	50	21
70 ft drop (approx), single fall at approx 75° F.	71	12	17

Summary confined to liners and overwraps only

Water-vapour Transmission of Packaging Materials

Materials and Methods

Earlier work has indicated that the most satisfactory evaluation of the water-vapour resistance of packaging materials was obtained after fabrication into packages (3)   Moreover, the experiment described above showed that the most desirable method of using a water-vapour barrier was as a liner inside the package   Hence, in the present work, all barriers (with one exception as noted below) were fabricated into carton liners.

The materials tested were different grades and plies of Cellophane, and various combinations of metal foil, scrim (a material similar to cheesecloth), kraft paper, glassine, Cellophane, Pliofilm, cellulose acetate, and vinylite. In addition, some of the materials were tested after waxing. Detailed descriptions of individual materials are given in Tables III, IV and V.

The materials were fabricated into pouch type liners, having outside dimensions of $5\frac{3}{8}$ by $6\frac{3}{4}$ in. high and inside dimensions of $4\frac{3}{8}$ by $6\frac{1}{4}$ in. high, suitable for use inside the chipboard carton which has already been described. The liners were opened, inserted into the cartons and partially filled with sawdust; then 73.5 gm. of anhydrous calcium chloride in a perforated P.T. Cellophane bag was added; this bag was surrounded by sawdust and the remainder of the liner was filled with sawdust.   The liner and cartons were then sealed, sodium silicate glue being used to make the carton closure.

The one exception to the above procedure was the material composed of scrim laminated to M.S.A.T. Cellophane, both sides being waxed with micro-

crystalline wax. This material was designed for overwrapping, and so was applied in this way. A double fold was made at the side seam, and completed packages were dipped in microcrystalline wax held at 170° F.

Five tests were done on each packaging material, six completed packages being used for each test. Six packages without calcium chloride and sawdust were used as a means of estimating the sorption of water vapour by the packaging materials. To estimate the sorption by the material used as an overwrap, a set of dummy packages of the same size as the chipboard cartons used was made, using metal in place of the chipboard box, to eliminate all absorbent material beneath the barrier.

One set of six packages was placed in a cabinet operating at 95° F and 100% relative humidity (high humidity cabinet, vapour-pressure differential approximately 42 mm. of mercury). Another set was placed in an alternating cabinet which operated at 80° F. and 100% relative humidity (vapour-pressure differential approximately 26 mm. of mercury) for 12 hr., and 120° F , 55% relative humidity (vapour-pressure differential approximately 48 mm of mercury), for the remaining 12 hr. of the day. The latter test was designed to give packages an opportunity to breathe.

The remaining three sets of packages were subjected to various treatments before the water-vapour penetration was determined in the high humidity cabinet. One set was stored at 140° F. (relative humidity about 6%) for one month, to evaluate the effect on the barrier of storage under hot dry conditions. To simulate very severe conditions of handling and transport, one set was cooled to minus 40° F. and dropped three feet, 20 times (five falls on each upright edge of the master container). After cooling to minus 40° F the remaining set was subjected to a vacuum of 20 in. of mercury for two hours, to reproduce conditions encountered in air transport Dropping and low pressure tests were performed using a master carton, which has already been described. The arrangement of test packages in the master container is shown in Fig. 1. It will be noted that, in the dropping test, two of the packages were buried in the interior of the master carton, being surrounded by dummy packages, while the remaining four were placed at the edges of the master carton.

To determine moisture gain, packages, as individual units, were weighed before insertion in the cabinets and at weekly intervals for four weeks.

Since packages are not normally stored in an atmosphere of 95° F., 100% relative humidity, it was desirable to be able to interpret water-vapour transmission rates in terms of temperate room conditions Hence, the transmission of three materials (Reynolds' Metal A–10, 300 M.S.A.T. Cellophane, and 300 M.S.T. Cellophane wax-coated 40 lb. per ream) was determined under the conditions existing in the laboratory, as well as in the high humidity cabinets. Packages were made up as previously described, and weighed at weekly intervals from August 1, 1945, to February 1, 1946.

Results

Water-vapour transmission rates are shown in Tables III, IV and V. To obtain these values, the weight of water vapour sorbed by the packaging materials (empty packages) at a given time was subtracted from the total increase in weight of the test packages, weighed at the same time. The slope

TOP

MIDDLE

BOTTOM

Fig. 1.   *Packages in master container, arranged for handling trials (packages under study marked, all others dummies).*

    ◯ *Dropping test, methods of application trials.*
    ● *Low pressure test.*
    □ *Dropping test, material trials.*

of the line showing weight gain per week of the package contents over a period of one month was then calculated assuming a straight line relation.

The average standard error for the transmission rates, except those obtained after the dropping test, was 0.24, hence rates must differ by 0.48 gm. per

TABLE III

SUMMARY OF WEIGHT CHANGES IN PACKAGES UTILIZING CELLOPHANE AS A LINER

Material	Thick-nesses	Seal	Sorption (gm.) by packaging materials held in high humidity cabinet	Water-vapour transmission (gm /week), high humidity cabinet, after treatments as follows·				Water-vapour trans-mission (gm./ (week), alternat-ing cabinet
				Untreated	One month at 140° F.	Subjection to low temp. and low pressure	Dropping* 20 times at −40° F.	
300 M.S.T.	Duplex	Crimp	1 84	0 89	10 78	1 66	6 82	1 44
300 M.S.A.T.	Duplex	Crimp	2 68	0 79	3 23	1 31	2 32	0 99
300 M.S.Y.T.	Duplex	Crimp	2 50	0 48	1 22	0 86	0 97	0 65
300 M.S.A.T.	Single	Crimp	1 44	1 46	8 83	2 75	4 92	1 30
300 M.S.A T.	Duplex	Crimp	2 68	0 79	3.23	1 31	2 32	0 99
300 M.S.A.T.	Triplex	Crimp	3 37	0 25	0 62	0 82	2 17	0 78
300 M.S.A.T.	Duplex	Flat	2 96	0 31	5 50	0 96	2 87	0 83
300 M.S.A.T.	Duplex	Crimp	2 68	0 79	3 23	1 31	2 32	0 99

* *Average for only two packages (see Table VI).*

week to be significantly different. As shown by Table VI, when most of the materials tested were subjected to dropping at −40° F., the four packages against the edges of the master container were fractured. For this reason, the water-vapour transmission rates obtained after the dropping were calculated from the gains of only the two packages buried in the interior of the master carton, and the standard error for these rates was larger, being 0.79. Keeping the above limits of accuracy in mind, it is possible to compare the protection offered by different materials under the conditions used.

Little difference was noted between the water-vapour transmission of untreated packages in the alternating and constant temperature cabinets, but any treatment simulating storage or transport markedly increased the rate of moisture gain (Tables III, IV, and V). After subjection to low temperature and p essure, the majority of packages showed a substantial increase in water-vapour penetration; however, the increase was no greater than that caused by the other handling tests, and no fractures in packaging material resulted from this treatment. This indicates that air transport should not damage packages extensively, provided air volumes inside the barriers are kept to a minimum.

When Cellophane was considered, it was apparent that M.S.Y.T. stock provided greater protection than M.S.A.T., which in turn was better than M.S.T. (Table III). The use of triplex bags gave maximum resistance to water vapour. A flat seal appeared to be generally more desirable than a crimp seal, although the latter was superior for excessively high storage temperatures.

TABLE IV

SUMMARY OF WEIGHT CHANGES IN PACKAGES USING PLAIN OR WAX-
COATED MATERIALS AS LINERS

Stock	Processing	Heat sealed	Sorption (gm) by packaging materials held in high humidity cabinet	Water-vapour transmission (gm /week), high humidity cabinet after treatment as follows				Water-vapour transmission (gm / week) alternating cabinet
				Un-treated	One month at 140° F	Sub-jection to low temp and low press	Drop ing* 20 times at −40° F	
40 lb kraft	Wax impregnated	(Adhesive sealed)	3 38	14 0‡	7 32‡	13 2‡	17 8‡	6 16
40 lb wet strength kraft	Wax coated†† 40 lb /ream	Flat	7 58	1 42	1 77	1 86	3 79	2 83
25 lb bleached glassine	Thermoplastic coated one side	Flat	1 94	4 97	†	6 98	15 66	4 61
25 lb bleached glassine	Wax coated†† 40 lb /ream	Flat	8 46	0 84§	1 19	1 25§	†	2 06
300 M S T Cellophane	None	Flat	2 28	1 85	33 0‡	2 41	10 06	1 50
300 M S T Cellophane	Wax coated†† 40 lb /ream	Flat	8 50	0 79	0 31	3 02	†	0 80
Laminated 300 M S T Cellophane	None	Flat	4 10	0 59	0 08	0 46§	1 76	0 66
Laminated 300 M S T Cellophane	Wax coated†† 40 lb /ream	Flat	3 57	0 24	0 06	0 08	0 89	0 04
Pliofilm	None	Flat	1 43	1 18	1 27	1 35	2 03	1 16
Scrim laminated to M S A T Cellophane waxed both sides	Applied as over wrap Package wax dipped (a) Scrim side in (b) Scrim side out	— —	0 18 0 75	0 17 0 02	0 39 0 22	0 13 0 39	0 19 0 17	0 11 0 14

* *Average for only two packages (See Table VI)*

† *Complete failure under these conditions*

‡ *Measurement for first two weeks only, complete failure*

§ *Averages for five packages only—one failure under these conditions.*

†† *Flexible wax composition.*

Wax-coating (40 lb per ream) various base stocks reduced the moisture penetration to less than one-half; but the transmission of all stocks was not reduced to a common value, i e , the more dense the base stock the lower the water-vapour transmission after waxing (Table IV). Wax-coating Cellophane and glassine reduced the moisture gain after ageing at 140° F , but did little to increase resistance to fracture at low temperature (Tables IV and VI). It will be noted that the material that was applied as an overwrap withstood dropping slightly better than other waxed materials. This was due to the fact that the wax on this material was more flexible than that on the coated materials, and

to the strength imparted to it by the scrim incorporated into the sheet. It must be borne in mind, however, that the transmissions reported after drop-

· TABLE V

SUMMARY OF WEIGHT CHANGES IN PACKAGES USING LAMINATED MATERIALS AS LINERS

Stock	Laminated to (wax laminated unless otherwise stated)	Heat sealed	Sorption (gm) by packaging materials held in high humidity cabinet	Water vapour transmission (gm /week) high humidity cabinet after treatment as follows				Water-vapour trans-mission, (gm / week) alternat-ing cabinet
				Un treated	One month at 140° F	Sub-jection to low temp and low press	Drop ing* 20 times at −40° I	
Scrim (Reynolds' A–50)	Kraft and alloyed lead foil with butvar coating (asphalt lam)	Flat and reinforced with cellu-lose tape	1 79	0 02	0 25	0 00	0 60	0 04
25 lb kraft	25 lb kraft, thermo plastic coated one side	Flat	2 04	8 92	29 26†	17 96†	15 55†	4 92
25 lb kraft	25 lb glassine thermo plastic coated on glassine	Flat	2 40	2 36	10 90‡	2 64	3 63	3 77††
White kraft	300 M S T Cello phane	Crimp	3 92	0 80	0 46	1 13	5 48	0 43
25 lb kraft	300 M S A T Cello phane	Flat	3 50	0 80	1 30	1 40	2 83	0 79
25 lb kraft	Cellulose acetate thermoplastic coated on acetate side	Flat	2 24	1 62	9 73	4 78	6 58	1 42
25 lb kraft (Reynolds' A–15)	Alloyed lead foil (asphalt lam) thermoplastic coat on foil	Flat	0 85	0 24	0 30	0 34	1 20	0 22
40 lb kraft (Reynolds' A–10)	Alloyed lead foil and Cellophane (asphalt lam)	Flat	2 15	0 00	0 13	0 10	0 97	0 13
25 lb glassine	25 lb glassine thermoplastic coated one side	Flat	2 08	1 24	1 14	1 59	1 92	3 78
25 lb glassine	300 M S I Cello phane	Crimp	1 03	0 77	0 24	0 99	4 95	0 49
300 M S T Cellophane	300 M S T Cellophane	Flat	4 10	0 59	0 08	0 46§	1 76	0 66
300 M S T. Cellophane	Aluminum foil	Crimp	1 43	0 05	0 37	0 35	1 43	0 19
Vinylite	Both sides of aluminum foil	Flat	2 06	0 00	0 00	0 00	0 00	0 00

* *Average for only two packages (See Table VI).*

† *Complete failure under these conditions.*

‡ *Measurements for first three weeks only, complete failure.*

§ *Average for five packages only, one failure under the conditions*

†† *Average for four packages only, two failures under these conditions.*

TABLE VI

ABILITY OF PACKAGES TO WITHSTAND 20 DROPS OF THREE FEET EACH AT −40° F. (−40° C.)

Stock	Processing	Packages unbroken—* (six tested)	Packages with water-vapour transmission comparable to that of interior packages
Scrim (Reynolds' A–50)	Laminated to kraft and foil with butvar coating	6	4
40 lb. kraft	Wax-impregnated	6	0
White kraft	Laminated to 300 M.S.T. Cellophane	6	1
Metal foil	Laminated to 300 M.S.T. Cellophane	6	0
Scrim laminated to M.S.A.T. Cellophane waxed both sides	Applied as overwrap. Package wax dipped. (a) Scrim side in (b) Scrim side out	6 6	1 0
Kraft (Reynolds' A–10)	Laminated to metal foil and Cellophane	5	0
25 lb. kraft (Reynolds' A–15)	Laminated to metal foil with thermoplastic coating	5	0
25 lb. kraft	Laminated to cellulose acetate	5	1
Vinylite	Laminated to both sides of aluminum foil	4	2
40 lb. kraft (wet strength)	Wax-coated 40 lb /ream	3	0
25 lb. kraft	Laminated to 25 lb glassine	3	0
25 lb. kraft	Laminated to 300 M.S.A.T. Cellophane	3	0
300 M.S.T. Cellophane	Laminated to 300 M.S.T. Cellophane	3	1

All other types had only two interior packages unbroken (see Tables III, IV, and V).

ping were based on the two interior packages only, and must be considered in conjunction with Table VI.

Laminated materials having metal foil as one layer provided greater protection than any other materials (Table V). The results also show that laminating two stocks produced marked reduction in the water-vapour transmission typical of either base sheet when used alone. The lamination of vinylite to metal foil illustrates that it is possible to produce a very water-vapour resistant package by combining a material that is not water-vapour resistant with one that is. Vinylite is not considered water-vapour proof and was utilized in this combination to provide strength and heat sealing properties.

Table VI shows the number of packages remaining unfractured after the dropping test, and indicates that the great majority of materials tested require protection from such treatment. Laminations utilizing scrim or kraft were less likely to fracture when roughly handled, but absence of a visible break' did not necessarily mean that the water-vapour transmission rate had not increased considerably.

Under room conditions, the water-vapour transmission rates of Reynolds' Metal A–10, 300 M.S.A.T. Cellophane, and 300 M.S.T. Cellophane wax-coated 40 lb. per ream were 0 00, 0 20, and 0 11 gm. per week, respectively. The foil barrier had an undetectable transmission rate in the high humidity cabinet (untreated packages, Table V), and this was also true under room conditions. However, for 300 M.S.A T. Cellophane and for wax-coated Cellophane, the ratio of the water-vapour transmission in the high humidity cabinet to the transmission under room conditions was 7 3 and 7 2, respectively.

Acknowledgments

References

1 LAVFRS, C G and PEARCE, J A   Unpublished data.
2 PEARCL, J A   Can J Research, F, 23 9-21. 1945.
3. WOODCOCK, A. H., CHAPMAN, M G , and PEARCE, J A   Can. J. Research, F, 23 : 109-116. 1945.

LIQUID AND FROZEN EGG

III. SOME FACTORS AFFECTING THE QUALITY OF STORED FROZEN EGG[1]

By Jesse A. Pearce[2] and Margaret Reid[2]

Abstract

Liquid from eggs of various qualities packaged in Reynold's Metal A-10 and liquid from Grade *A* eggs in wax paper with and without added ice was frozen at −40° F. and stored at 10°, 0°, and −10° F. for 12 months. Examination of baking properties and changes in pH, fluorescence, and reducing sugar content indicated the desirability of using liquid from Grade *A* eggs, although liquid from Grade *C* and cracked eggs may also be satisfactory; and of limiting the storage period for frozen egg, stored at these temperatures, to about six months. It was also desirable to allow the frozen egg to age for a month or two before use; and to use a highly moisture resistant barrier at all storage temperatures, although the wax paper and ice combination may be satisfactory at 0° and −10° F. Reducing sugar content decreased with an increase in the number of bacteria and, in addition, this measurement appeared to be a good indication of the quality of liquid and frozen egg.

Introduction

Since the production of eggs is seasonal, it is frequently necessary to carry large stocks for six months of the year or longer. The perishable nature of this commodity demands attention to the manner in which it is stored. While eggs in the shell can be held for short periods, some other method of preservation is desirable. Preserving eggs by removing them from the shell, mixing the yolk and white, and freezing has been an important commercial process for many years. During the war years, a large proportion of Canada's eggs were exported in the dried form; nevertheless, about five million pounds was frozen for use by bakery and other trades, exclusive of the quantities frozen for subsequent drying. However, only limited information is available to describe the keeping quality of the frozen product and the chemical changes occurring during its storage.

It seemed advisable before beginning the studies described in this paper to consider some of the changes likely to occur in eggs. An examination of frozen egg stored for six years at a temperature of 0° to −5° F. showed that if eggs of good quality were frozen, the odour of the product did not change but, if poor quality eggs were frozen, the initial putrid odour seemed to intensify (12). Although there appeared to be an increase in ammoniacal nitrogen as eggs became inedible (15, pp. 223-234), it seemed unlikely that this test would prove useful (15, pp. 260-261) and this was substantiated by preliminary work in these laboratories. As eggs deteriorate there is an increase in formic, lactic, and acetic acid content (5); therefore, measure-

---

ments of pH changes seemed desirable.   During work on dried eggs, it was observed that the fluorescence of extracts of the powder was related to the quality (9).   Further work has indicated that fluorescence development may be attributed, in part, to a reaction between reducing sugars and proteins (1, 6, 7).   Therefore, two modifications of this fluorescence test were applied to liquid eggs (10).   It also appeared desirable to measure changes in reducing sugar content of the eggs, since this component has an important effect on the keeping quality of dried eggs (13) and since reducing sugars might disappear if the foregoing reaction occurred   In addition, sugar might also be removed by microbial growth (15, pp 233-234).   Much of the commercial frozen egg is used by the baking trade, and, for this reason, sponge cakes were believed desirable as tests of baking quality.

Materials and Methods

The storage experiment utilized material described in an earlier paper (10, Table IV).   In brief, liquid from fresh Grade A eggs, from Grade A eggs held for 16 hr. at 80° F. in sterile glass containers, from Grade $C*$ eggs, from cracked eggs, from musty eggs, and from "eight-day" incubator reject eggs (for grade descriptions, see (2)) was poured into moulds containing about four litres, frozen within 16 hr. in a room operating at −40° F. and held at temperatures of 10° (+ 2°, − ½°), 0° (± 1°), and − 10° (± 1°) F. for a period of one year.   Samples were examined before and after freezing, and after 3, 6, and 12 months' storage.   The liquid from fresh Grade A eggs was packed in Reynold's Metal A-10, a highly moisture vapour resistant material (16), plus a Fourdrinier kraft, B-Flute carton; in waxpaper (40 lb. kraft, waxed to 50 lb.) plus the carton with ice cubes (about 2 cu. in. in volume) inside the carton around the wrapped egg; and in wax-paper and carton without added ice.   All other samples were packed in Reynold's Metal A-10 and cartons.

The analyses included measurements of pH and reducing sugar (4, pp. 416 and 438) on whole egg liquid; fluorescence of whole egg liquid, using a modification of a technique applied to egg powder (3); and baking volume and foaming volume measurements on whole egg liquid (11).   In one portion of the study, reducing sugar content and pH of liquid egg, before and after freezing, were compared with the viable bacterial count (14).

In the initial stages of the study, pH, reducing sugar and fluorescence measurements were made on sera removed from the frozen egg by the chloroform treatment described earlier (10).   It was possible to collect enough sera for all measurements on samples up to and including the three-month storage period.   At the six-month storage period, only enough serum was separated from any one sample to permit fluorescence evaluation.   The fluorescence changes up to the six-month sampling have been discussed (10) and at the 12-month storage period, the structure of the frozen egg had so changed that no sera could be separated.

* Eggs may be graded as C because of dirty shells or because of poor quality before the candling lamp (2); those used in this study were selected for poor quality.

Results

Effect of Bacterial Growth Before Storage on Reducing Sugar Content and pH

The relations between bacterial growth, reducing sugar content, and pH changes in frozen and unfrozen liquid egg are shown in Table I. Bacterial growth was most rapid in egg yolk and least rapid in egg white but both whole egg and egg yolk attained about the same bacterial populations after holding for 48 hr. at 80° F. Slight bacterial growth in liquid from whole egg and egg

TABLE I

THE RELATIONS BETWEEN REDUCING SUGAR CONTENT, pH, AND BACTERIAL GROWTH IN FROZEN AND UNFROZEN EGG

Material and treatment	Viable bacteria at 37° C.		Reducing sugar, %		pH	
	B.F.*	A.F.*	B.F.	A.F.	B.F.	A.F.
Whole egg						
48 hr. at 30° F.	$<1.0 \times 10^2$	1.0×10^2	0.32	0.28	7.7	8.1
24 hr. at 80° F. and						
24 hr. at 30° F.	4.2×10^4	2.2×10^4	0.29	0.26	7.7	8.0
48 hr. at 80° F.	1.1×10^9	1.8×10^8	0.05	0.05	5.9	6.0
Egg white						
48 hr. at 30° F.	$5 3 \times 10^3$	2.0×10^3	0 32	0.33	8.9	8.9
24 hr. at 80° F. and						
24 hr. at 30° F.	$4 6 \times 10^3$	7.7×10^3	0.32	0 32	8 5	8.3
48 hr. at 80° F.	7.0×10^4	2.2×10^5	0 29	0 25	8.2	8.3
Egg yolk						
48 hr. at 30° F.	$5 0 \times 10^2$	5.0×10^2	0 24	0.26	6.4	6.4
24 hr. at 80° F. and						
24 hr. at 30° F.	$1 4 \times 10^6$	1.4×10^6	0 24	0.27	6 4	5.9
48 hr. at 80° F.	5.9×10^8	3.0×10^8	0 04	0.06	5.0	4.9

B.F.—Before freezing.   A.F.—After freezing.

white had a more marked effect on the sugar content than on pH, while, for separated yolk, fairly excessive bacterial growth had little effect on either measure. Freezing appeared to effect slight reduction in the bacterial content of some samples, and to have little effect on pH or reducing sugar content of liquid from egg white, but caused an apparent reduction in the reducing sugar content of liquid from whole egg and an apparent increase in the reducing sugar content of liquid from yolks.

Quality Changes During Storage

Baking volume, foaming volume, pH, and fluorescence measurements on the whole egg liquid were of little value in differentiating between many of the stored samples and were of less value in differentiating between storage temperature and between method of packaging. Baking or foaming volume measurements were of value in demonstrating the effect of storage time.

Volume measurements on sponge cakes made from the various types of liquid egg showed that musty eggs and incubator rejects were less suitable

than the other types of egg (Fig. 1). Liquid egg before freezing or frozen egg stored for three months gave larger cakes than egg just after freezing. At the six-month sampling, the baking volume was slightly less than that at the three-month sampling and at the twelve-month sampling it was markedly
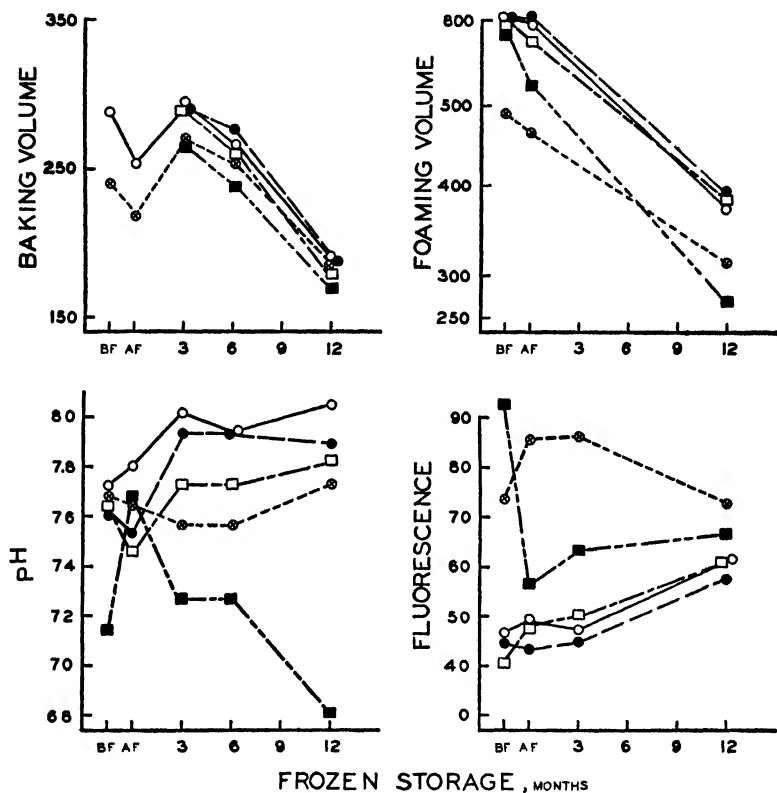

FIG. 1. *Effect of freezing and frozen storage on baking volume (ml.), foaming volume (ml.), pH, and fluorescence of liquid from eggs of varying quality.* O *Grade A.* ● *Grade C.* □ *Cracks.* ■ *Musties.* ⊕ *Incubator rejects.*

less. This reduction in the baking volume of egg when freshly frozen is known to occur in commercial practice and, in this state, the product is known as "green egg." Many concerns handling frozen egg prefer to let it age for a short period before releasing it.

Foaming volume measurements also showed that musty eggs and incubator rejects were less likely to be satisfactory in baked goods (Fig. 1). After 12 months' storage, egg held at 10° F. averaged 25 ml. less than the samples held at 0 or −10° F. Egg wrapped in Reynold's Metal A-10 and stored at 10 and 0° F. had foaming volumes 30 ml. greater than when wrapped in waxed paper (with or without added ice): at −10° F. no difference was evident.

Musty eggs were generally more acidic than incubator rejects, which in turn were more acidic than all other types of egg studied (Fig. 1). All types

of frozen egg, except that prepared from musty eggs, tended to become more alkaline as the storage time increased. Liquid from musty eggs increased in pH markedly during the freezing period and then decreased rapidly as storage progressed. Liquid from Grade *A* eggs suffered smaller pH increases when stored at −10° F. than when stored at 0° and 10° F. (Table II).

TABLE II

SOME EFFECTS OF TEMPERATURE ON THE QUALITY OF FROZEN EGG

Criteria	Type of eggs	Tempera-ture, °F.	Storage time				
			B.F.*	A.F.*	3 Mos.	6 Mos.	12 Mos.
pH	Grade *A*	10			8 02	8.03	8.11
		0	7.72	7.80	8.08	7.87	8.08
		−10			7.92	7.90	7.95
Fluorescence	Incubator rejects	10			83.0	—	60 1
		0	73.5	85.4	88.0	—	76.1
		−10			86.0	—	80.9
	Musty	10			64.0	—	75.9
		0	92.8	56.6	62.0	—	62.0
		−10			64 0	—	61.4

B.F.—Before freezing. A.F.—After freezing.

The fluorescence of material from Grade *A*, Grade *C*, and cracked eggs was generally lower than that of musty eggs and incubator rejects and showed a general increase through freezing and frozen storage, with the fluorescence value of Grade *A* and cracked eggs somewhat higher than the values for Grade *C* eggs (Fig. 1). The high fluorescence values of liquid from Grade *A* and cracked eggs when compared to those of liquid from Grade *C* eggs may reflect differences between measurements directly on the melange and measurements on the serum (10), or may be only a reflection of the limited number of samples of eggs used. In an earlier study, more comprehensive on this point, liquid from Grade *C* eggs, on the average, resulted in powders with fluorescence values higher than those of liquid from Grades *A* and *B* eggs (8).

Incubator rejects gave a product that increased in fluorescence during freezing and decreased in this attribute during frozen storage, while musty eggs gave a product that decreased in fluorescence during freezing and increased during frozen storage (Table II). The decreases for reject egg and the increases for musty egg were more rapid at + 10° F. than at 0° and −10° F. While the fluorescence changes in the liquid from incubator rejects are at present unexplainable, the fluorescence and pH changes in musty egg may be related to commercial observations. If pails of liquid have a musty odour and are allowed to stand for about 24 hr. at 30° F., the musty odour will disappear. It is possible that the volatile products are acid in nature and

highly fluorescent. However, loss of these volatiles did not improve baking quality. An attempt is being made, in these laboratories, to isolate the possible acid, fluorescing volatiles.

Measurements of the reducing sugar content appeared to be of greater value than the foregoing and are shown in more detail (Fig. 2). These measure-
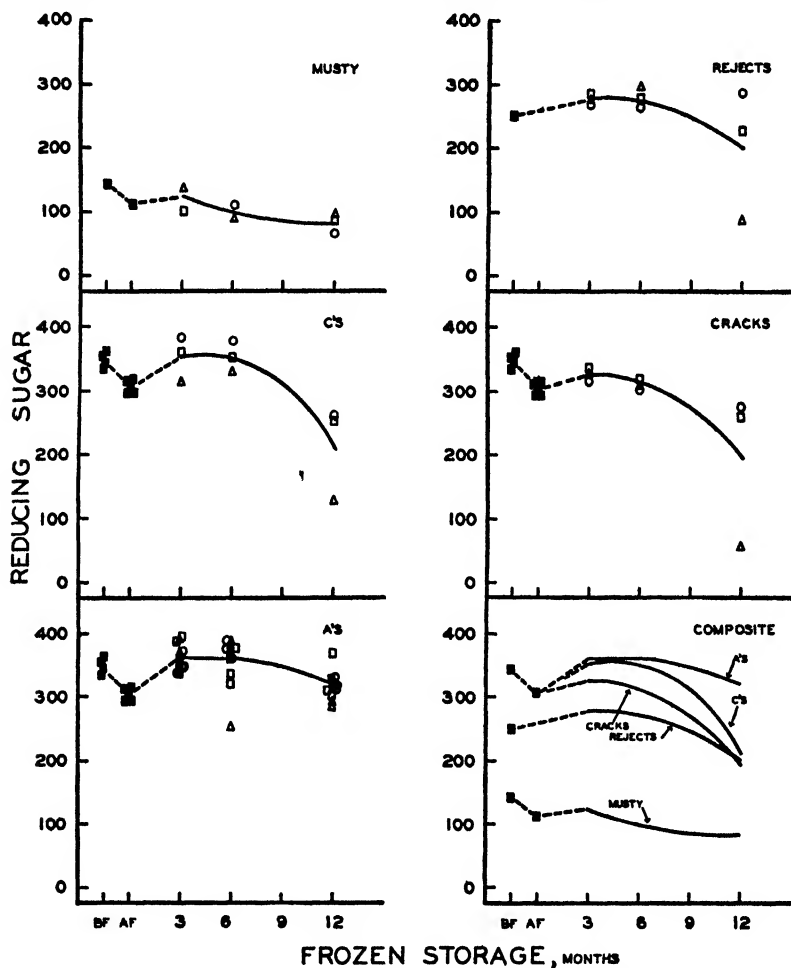
FIG. 2. *Effect of freezing and frozen storage on reducing sugar content (% × 1000) of liquid from eggs of varying quality.* ■ *Values before (B.F.) and after freezing (A.F.).* △ *Storage at 10° F.* □ *Storage at 0° F.* ○ *Storage at −10° F.*

ments showed greater loss in sugar content as the storage temperature increased; as shown by the points in the figure, however, only mean changes for the various types of egg are given by the curves. The apparent decrease in reducing sugar content after freezing, observed in the preliminary study, was again noted here. This was followed by an increase after three months' storage but after six months' storage reducing sugar again decreased. These changes corresponded in general with the changes in baking volume.

The reducing sugar content was greatest for liquid from Grade A eggs and, decreased for liquid from the other types in the following order: Grade C, cracked, incubator reject, and musty eggs. The reducing sugar values reported here for fresh liquid egg are in general agreement with some values reported in the literature, but lower than others (15, pp. 230 and 249). Measurements of reducing sugar appear to be a good indication of egg quality and they can be readily performed in a plant laboratory or by local consulting laboratories.

TABLE III

EFFECT OF PACKAGING METHOD ON FREEZER BURN IN FROZEN EGG STORED 12 MONTHS

Packaging method*	Approximate depth of dehydrated surface, in., at various temperatures		
	10° F.	0° F.	−10° F.
All samples in Reynold's Metal A-10	0	0	0
Liquid from Grade A eggs in wax paper plus ice chunks	>1	0	0
Liquid from Grade A eggs in wax paper only	½	¼	⅛

* All further enclosed in kraft carton.

The effect of freezer burn on the frozen blocks is described in Table III. The greatest general protection was afforded by the Reynold's Metal wrap. The addition of chunks of ice reduced freezer burn at storage temperatures of 0° and −10° F. but appeared to accelerate freezer burn at + 10° F. The latter effect may be attributable to differences in specific heat of the ice and the liquid egg, resulting in transfer of moisture from the blocks of frozen egg to the ice as the temperature of the storage room and its contents varied around the controlled temperature, 10° (+ 2°, −½°) F.

Acknowledgments

References

1. BATE-SMITH, E. C. and HAWTHORNE, J. R.   J. Soc. Chem. Ind. 64 : 297-302. 1945.
2. DEPARTMENT OF AGRICULTURE, CANADA.   Regulations respecting the grading, packing and marking of eggs. Ottawa. 1940.
3. FRYD, C. F. M. and HANSON, S. W. F.   J. Soc. Chem. Ind. 63 : 3-6. 1944
4. HAWK, P. B., BERGEIM, O., OSER, B. L., and COLE, A. G.   Practical physiological chemistry.   11th ed.   P. Blakiston's Son & Co., Inc., Philadelphia. 1937.
5. LEPPER, H. A., BARTRAM, M. T., and HILLIG, F.   J. Assoc. Official Agr. Chem. 27 : 204-223. 1944.
6. OLCOTT, H. S., DUTTON, H. J., and EDWARDS, B. G.   Ind. Eng. Chem. 37 : 1119-1126. 1945.

7. PEARCE, J. A. and BRYCE, W. A.   Proc. Inst. Food Tech.   In press.
8. PEARCE, J. A., REID, M., METCALFE, B., and TESSIER, H.   Can. J. Research, F, 24 : 215-223.   1946.
9. PEARCE, J A. and THISTLE, M. W.   Can. J. Research, D, 20 : 276-282.   1942.
10. REID, M.   Can J. Research, F, 24 : 130-135.   1946.
11. REID, M. and PEARCE, J. A.   Can J. Research, F, 23 : 239-242.   1945.
12. SCHNEITER, R., BARTRAM, M. T., and LEPPER, H. A.   J. Assoc. Official Agr. Chem. 26 : 172-182.   1943.
13. STEWART, G. F., BEST, L. R., and LOWE, B.   Proc. Inst Food Tech. 77-89.   1943.
14. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E.   Can. J. Research, D, 21 : 1-7.   1943.
15. WINTON, A. L. and WINTON, K B   The structure and composition of foods.   Vol. III. John Wiley & Sons, Inc., New York.   1937.
16. WOODCOCK, A. H., CHAPMAN, M G , and PEARCE, J. A.   Can. J. Research, F, 23 : 109-116.   1945.

A STUDY OF METHODS FOR ASSESSING RANCIDITY IN LARD

By G. A. Grant and H. J. Lips

A STUDY OF METHODS FOR ASSESSING RANCIDITY IN LARD[1]

By G. A. Grant[2] and H. J. Lips[3]*

Abstract

Lard from 26 sources was stored in glass jars at 26 7° C (80° F) until definitely rancid. Spoilage was evaluated at two-week intervals by chemical tests and odour ratings. Correlation coefficients between odour scores and the logarithms of chemical test values were: iodometric peroxide, − 90; alpha-dicarbonyl test, − 85; Stamm test − 82; Kreis test −.81; ferrometric peroxide, −.80; fluorescence, 79; free fatty acids, −.10. Association between chemical measurements was greatest between alpha-dicarbonyl and iodometric peroxide values ($r =$ 97). As peroxides are not thermostable, the measurement of the stable alpha-dicarbonyl compounds, although less precise, is considered the best available chemical method for assessing rancidity.

Introduction

Recent increases in production and export of Canadian lard have focused attention on the perishability of the product. As part of a program to improve the stability and general quality of lard, a study was made of available chemical methods for detecting rancidity. A number of these methods and their association with odour ratings are described in the present paper.

Methods

The exact measurement of rancidity development in fats by any one procedure is difficult. This is due to the diversity of the reactions producing rancidity, e.g., atmospheric oxidation and the action of micro-organisms and enzymes. Taste and smell, among the least sensitive of the senses, have been widely used as criteria of rancidity but since odour and taste judgments are difficult to reproduce, it is desirable to employ chemical or physical measurements, which are reproducible and can be calibrated against the results of odour or taste panels.

Considerable uncertainty exists concerning the relative merits of the chemical tests and only those showing promising results from preliminary trials were selected for the present study. These included determination of peroxide oxygen, Kreis, Stamm, alpha-dicarbonyl, and fluorescence values.

Peroxide Oxygen Content

Several methods have been proposed for determining peroxides in fats (1, 5, 6, 11, 22). A modification (6) of an iodometric method (10) and a modification (11) of a ferrometric method (1) were selected. The iodometric is

simpler than the ferrometric procedure but the latter has been reported to be more sensitive.

A previous study on chicken fat indicated that peroxide oxygen content as determined by the iodometric procedure was 20% less than the actual content (2) because iodine was absorbed by the unsaturated fatty acids. As lard has an appreciably lower iodine number than chicken fat, it was assumed that this correction was not necessary. Lea's original practice (10) of reporting peroxide oxygen as millilitres of 0 002 N sodium thiosulphate is employed in this investigation for the iodometric procedure. The peroxide content by the ferrometric method (11) is reported as milliequivalents of peroxide per kilogram of fat.

Kreis Value

Of the several modifications of the Kreis test (9) one (20) was selected because of its simplicity and because the colour was developed in a single phase system. The colour intensity has been found proportional to the concentration of fat (21), increasing with a decrease in fat concentration. For this investigation the same concentration of fat, 1 gm. in 10 ml., was adopted for all determinations, dilution, if necessary, being made in the coloured solution. The Evelyn photoelectric colorimeter (3) was used for all colorimetric procedures. The colour intensity of the Kreis test was determined using a No. 540 mμ Rubicon filter, and reported as extinction coefficients according to the equation $E = \dfrac{2 - \log G}{C}$, where G is the corrected galvanometer reading and C is the concentration of fat in grams per millilitre of final solution. Although this value has little physical significance it is suitable for purposes of comparison.

Alpha-dicarbonyl and Stamm Values

Many tests for rancidity depend on the presence of aldehydes and ketones in the oxidized fat. Of the many methods for the detection of aldehydes and ketones in rancid fat (4, 8, 12, 16, 17), two (12, 17) were chosen for further study.

The Stamm method (17) used arbitrary standards to measure the colour developed, and was not sensitive to small changes in colour intensity. Use of a colorimeter was believed desirable to obtain accurate comparison between samples. The reagent was prepared by heating 0.5 gm. of s-diphenyl-carbazide in 100 ml. of tetrachloroethane until it dissolved, cooling rapidly, and filtering in a darkened room. It was then stored in a brown reagent bottle. Heating 1 0 gm. of fat with 10 ml. of reagent in a graduate for 3 0 min. at 100° C., cooling rapidly, and reading immediately in the Evelyn colorimeter employing a 580 mμ filter was found to be satisfactory. The results are reported as extinction coefficients.

The alpha-dicarbonyl method (12) was also modified for use with the Evelyn colorimeter. Preliminary test solutions containing 1.0 gm. of fat, 1.0 ml. of 30% potassium hydroxide solution, and 9.0 ml. of ethanol heated for 20 min. at 60° C., demonstrated differences between fresh and rancid lard.

However, the solutions were not suitable for reading in the colorimeter, as they separated into two phases. Employing stronger potassium hydroxide solution or heating for one hour failed to produce a single phase system, but with the use of 30 ml. of alcohol the solutions remained clear and in a single phase.

To investigate the effect of fat concentration on the extinction coefficient, 0 5, 1 0, and 3 0 gm of lard were heated with 3 0 ml of potassium hydroxide solution and 32 ml of purified ethanol   The solution containing 3 0 gm of lard became cloudy and had to be filtered   The ethanol was freed of aldehydes and ketones by refluxing with calcium oxide, distilling, shaking with 2,4-dinitrophenylhydrazine and redistilling   The results are given in Table I. The extinction coefficient decreased with an increase in fat concentration and 1 0 gm. of fat showed the largest difference between fresh and rancid lard.

TABLE I

THE EFFECT OF FAT CONCENTRATION ON EXTINCTION COEFFICIENTS IN THE ALPHA-DICARBONYL TEST

Weight of sample gm,	Extinction coefficients	
	Fresh	Rancid*
0 5	3 2	7 1
1 0	0 9	5 4
3 0	0 9	3 7

* The peroxide oxygen content of the rancid sample was
14 ml of 0 002 N thiosulphate per gm

The effects of temperature and time of heating were also investigated Solutions were heated for 30 min at 60° C and 100° C and for 30, 60, and 90 min. at 80° C. The results are shown in Tables II and III   Raising the

TABLE II

THE EFFECT OF TEMPERATURE AFTER HEATING FOR 30 MIN ON THE ALPHA-DICARBONYL EXTINCTION COEFFICIENTS

Temperature, °C	Extinction coefficients	
	Fresh	Rancid
60	0 2	9 4
80	0 7	8 4
100	0 4	9 1

temperature from 60° to 100° C. had little effect on the extinction coefficient, but increasing the time of heating resulted in a higher value.   This effect was more pronounced in fresh lard than in the rancid samples.

The procedure adopted was as follows. One gram of fat was weighed into a glass-stoppered graduate and 3 ml. of 50% potassium hydroxide solution and 30 ml. of purified ethanol were added. The solution was heated for 30

TABLE III

Effect of time of heating at 80°C on alpha-
dicarbonyl extinction coefficients .

Time, min	Extinction coefficients	
	Fresh	Rancid
30	0 72	8 4
60	1 70	8 9
90	2 12	9 2

min. at 80° C., then allowed to cool, made up to 35 ml. with alcohol, and read in an Evelyn colorimeter using a 420 mμ filter. The results were reported as extinction coefficients.

Fluorescence

Examinations of lard by ultra-violet light have been reported (13, 19, pp. 90-91). A method (13) using the Coleman photofluorometer standardized with quinine sulphate as previously described (14) was investigated. Fluorescence values are reported as photofluorometric readings of the lard solution minus blank solvent readings.

From previous work on butterfat (7) it was indicated that the type of solvent and concentration of fat might have a marked influence on the fluorescence values. To study the effect of organic solvents on fluorescence of fresh and rancid lard, 1 0 gm of lard was dissolved in 10 ml. of various solvents, slightly warmed, mixed thoroughly, and read on the photofluorometer. Petroleum ether, xylene, dioxane, benzene, and ethylene dichloride were studied, as the previous work had indicated a better differentiation between rancid and fresh fat with these solvents. The results are given in Table IV. The rancid lard gave lower fluorescence values with all solvents.

TABLE IV

The effect of organic solvents on the fluorescence
of fresh and rancid lard

Solvent	Fresh	Rancid
Petroleum ether	14 0	12 0
Xylene	19 5	12 0
Dioxane	16 5	8 7
Benzene	16 0	8 0
Ethylene dichloride	15 5	11 5

Greater differences were observed with dioxane, benzene, and xylene than with petroleum ether or ethylene dichloride. Xylene was chosen for further study as it gave the lowest blank reading.

To study the effects of concentration, 1- to 5-gm. samples of fresh or rancid lard were dissolved in 10 ml. of xylene. The results are given in Table V.

<div align="center">

TABLE V

THE EFFECT OF CONCENTRATION ON THE FLUORESCENCE
OF FRESH AND RANCID LARD

</div>

Weight, in gm per 10 ml. of solvent	Fluorescence value	
	Fresh	Rancid
1	13 7	8 7
2	27 5	12 2
3	37 2	15 0
5	Over 100	16 0

An increase in fat concentration gave an increase in fluorescence values. This increase was of greater magnitude with fresh lard than with the rancid samples. As the fluorescence changes between the fresh and rancid lard were not similar with dilution, an empirical method was adopted to ensure comparability. One gram of fat was weighed into a 10 ml. glass-stoppered graduate and 10 ml. of xylene added. The mixture was shaken until the fat was completely dissolved and then read in a Coleman photofluorometer.

Odour Tests

Rancidity in the lard was assessed by a 10 member panel and scored on the following basis: 10, excellent, odour fresh or absent; 8, good, no rancid odour; 6, fair, slight rancid odour; 4, poor, odour definitely rancid; 2, bad, odour very rancid; 0, unapproachable. Odours that could be classified as burnt, tanky, or otherwise objectionable, but not rancid, were given a rating of 7 on this scale.

Materials and Procedure

Samples of all types of lard manufactured in Canada were received from 26 Canadian packing plants and stored in half-pint glass jars at 26.7° C. Ten samples were put into storage every two weeks and sampled at two-week intervals. This ensured differences in level of rancidity, and convenience in the number of samples coming out of storage at any one time. Samples for odour tests were removed and the remainder melted on a steam-bath and mixed thoroughly to provide material for the objective tests. If all the tests were not completed on the day of sampling the material was stored at −40° C.

Results

The association between objective tests and the odour test was assessed by computing simple correlation coefficients. For predicting odour scores from objective test values, the correlation coefficient must be highly significant and attain a value of .8 to .9. A small scatter around the regression line is also desirable and this was assessed by computing the error of estimate. The coefficients of correlation with their errors of estimate and prediction equations between odour score and the logarithms of the objective tests are given in Table VI.

TABLE VI

THE CORRELATION COEFFICIENTS, PREDICTION EQUATIONS, AND ERRORS OF ESTIMATE BETWEEN ODOUR SCORE AND LOGARITHMS OF THE OBJECTIVE TEST DATA

Quantities correlated with odour score:	Degrees of freedom	Correlation coefficients	Prediction equations	Errors of estimate
Alpha-dicarbonyl	204	− 85	$y = 8\ 48 − 2\ 78x$	0 83
Iodometric peroxide oxygen	160	− 90	$y = 7\ 63 − 1\ 81x$	0 70
Ferrometric peroxide oxygen	204	− 80	$y = 8\ 30 − 1\ 52x$	0.94
Kreis	183	− 81	$y = 10\ 0 − 2\ 73x$	0 54
Stamm	204	− 82	$y = 7\ 67 − 2\ 01x$	0 91
Free fatty acids	108	− 10	—	—
Fluorescence	191	.79	$y = 5\ 53 + 6\ 63x$	0 96

A decrease in odour score was associated with an increase in all the objective values, except the fluorescence measurements, which gave a corresponding decrease. Of the objective tests correlated with rancidity as assessed by odour scores, the alpha-dicarbonyl values and peroxide oxygen determined by the iodometric procedure gave the highest associations. The Kreis, Stamm, ferrometric peroxide oxygen, and fluorescence values were all about equally associated with odour score. The sensitivity of the tests as assessed by the regression coefficients showed the ferrometric peroxide oxygen to be most sensitive. However, this test and the fluorescence measurement had the two largest errors of estimate. The fluorescence values, although highly associated with odour score, had regression values too high to enable prediction of odour scores lower than 5.0 (Fig. 6), the fluorescence values being almost nil at this point. This indicates that fluorescing substances were almost completely destroyed when the lard had just become rancid. The free fatty acid values were not significantly associated with odour scores.

Further details of the association between objective tests and odour scores are shown in Figs. 1 to 6. The equations for the relations shown in the figures are given in Table VI. It is evident from Figs. 1 to 6 and Table VI that a slightly rancid lard (odour score of 6) corresponded to the following objective test values: alpha-dicarbonyl value, 7.8; iodometric peroxide oxygen, 7.8; ferrometric peroxide oxygen, 31.6; Kreis value, 28.8; and Stamm value 6.8. The regression lines of the objective tests are shown in Fig. 6. The two
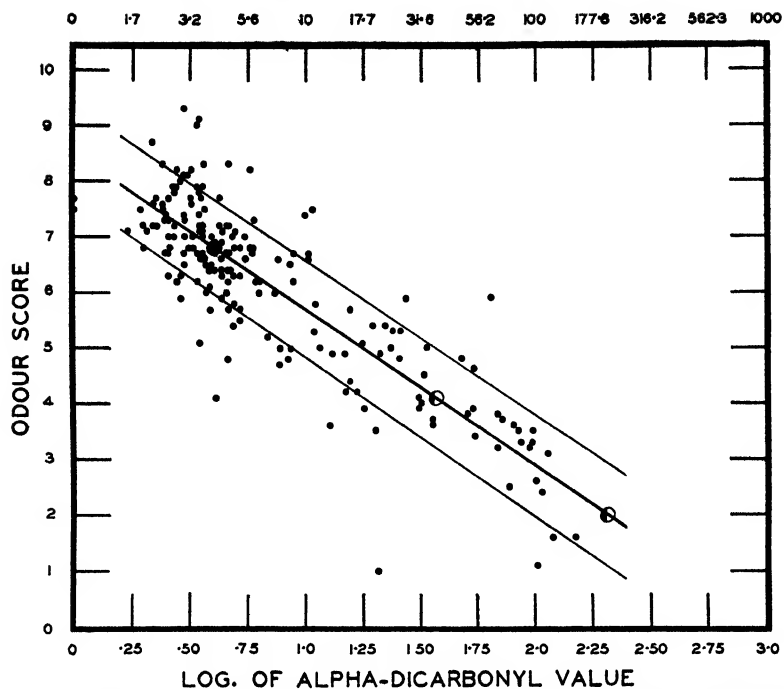
FIG. 1. *Relation between odour score and alpha-dicarbonyl value on development of rancidity in lard stored at 80° F.*
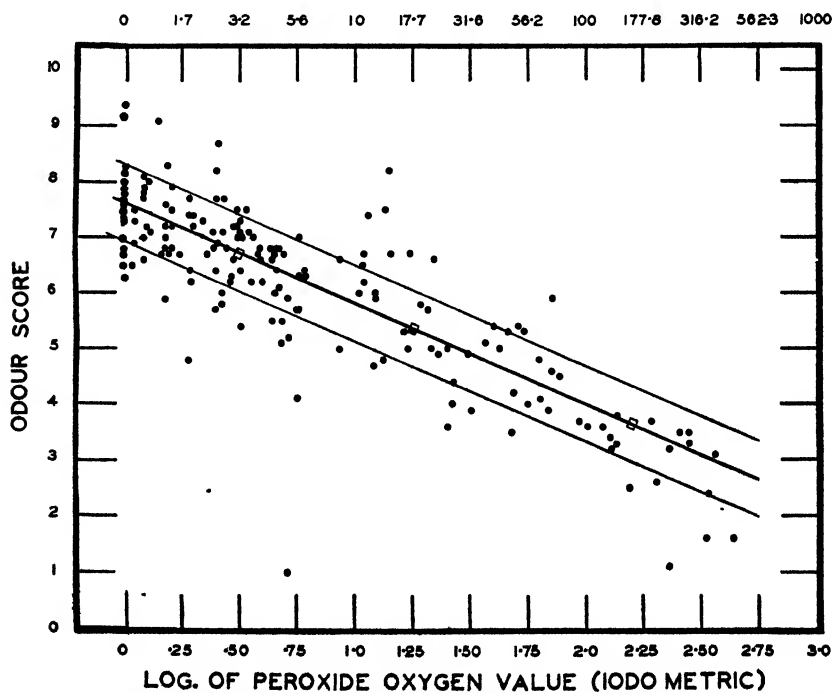

FIG. 2. *Relation between odour score and iodometric peroxide oxygen content on develop*
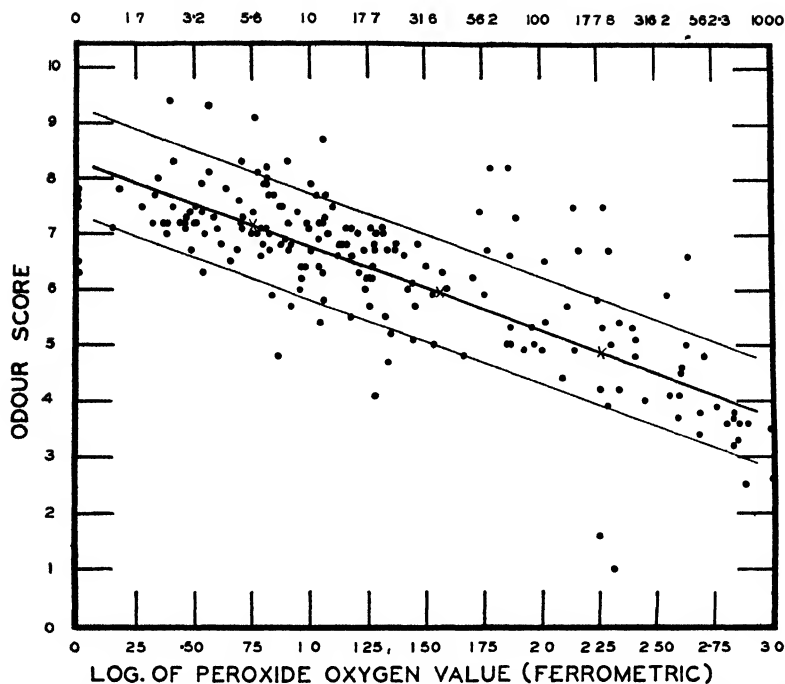
FIG 3   *Relation between odour score and ferrometric peroxide oxygen content on development of rancidity in lard stored at 80° F*
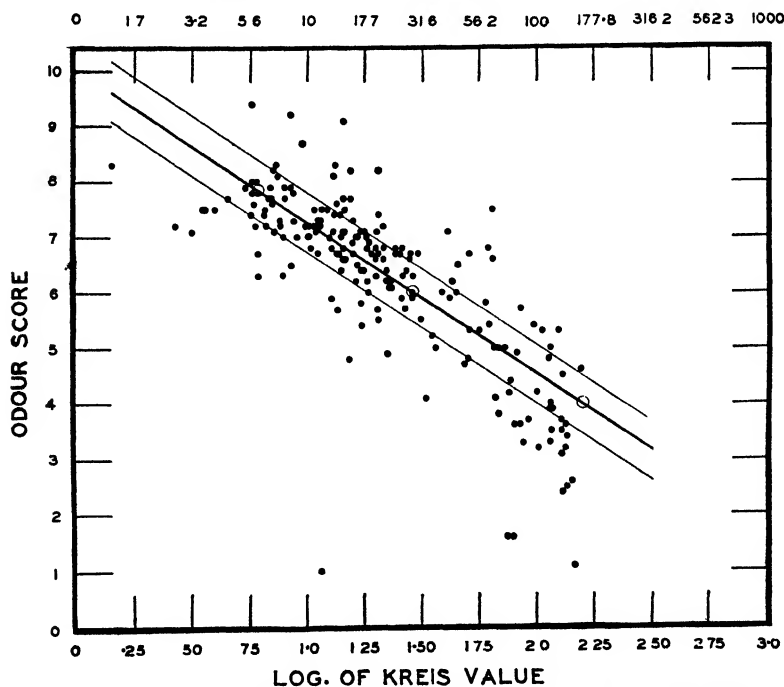

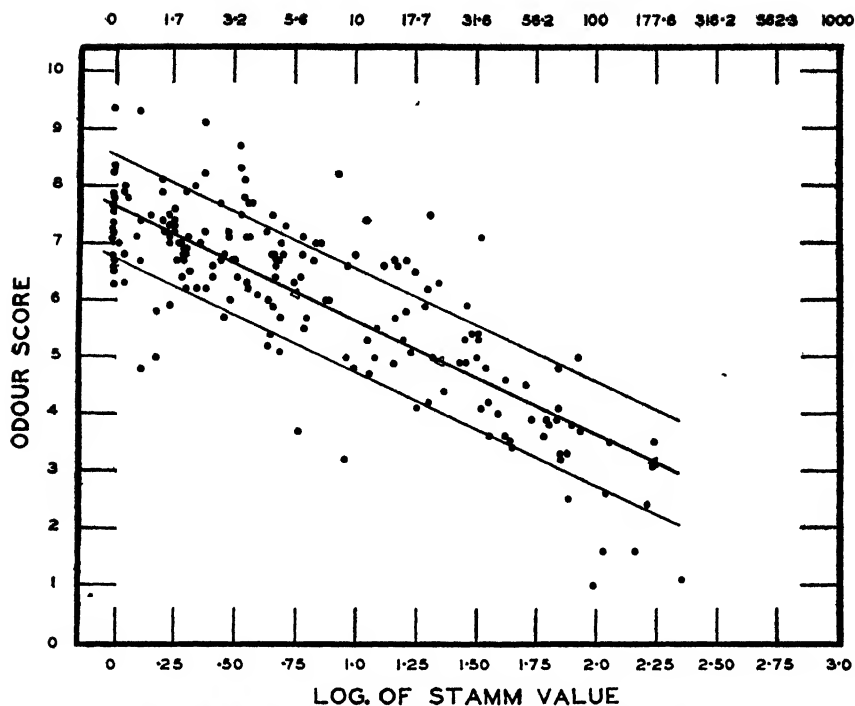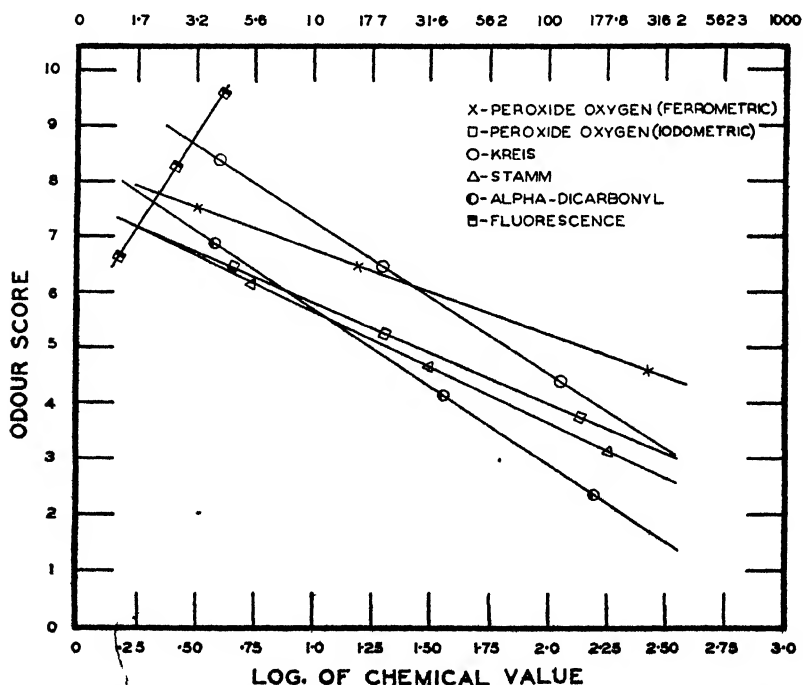FIG. 4.   *Relation between odour score and Kreis value on development of rancidity in lard stored at 80° F.*

FIG. 5.   *Relation between Stamm value and odour score on development of rancidity in lard stored at 80° F.*

FIG. 6.   *Relation of regression lines of the objective tests and odour score in lard which developed rancidity on storage at 80° F.*

peroxide methods gave the same slope. The Kreis and alpha-dicarbonyl measurements also showed equal slopes, but of greater magnitude than those of the peroxide values.

Interrelation of Chemical Measurements

The interrelation of the chemical measurements was assessed by computing simple correlation coefficients (Table VII) Of the methods investigated, the

TABLE VII

Simple coefficients of correlation between objective tests on lard that attained statistical significance

Quantities correlated	Degrees of freedom	Correlation coefficients
Alpha-dicarbonyl value with		
Iodometric peroxide oxygen content	204	0 97**
Ferrometric peroxide oxygen content	204	0 86**
Stamm value	204	0 60**
Logarithm of alpha dicarbonyl value with		
Kreis value	198	0 89**
Iodometric peroxide oxygen content with		
Stamm value	204	0 62**
Ferrometric peroxide oxygen content	204	0 84**
Logarithm of iodometric peroxide oxygen with		
Logarithm Kreis value	198	0 90**
Ferrometric peroxide oxygen content with		
Stamm value	204	0 60**
Logarithm of ferrometric peroxide oxygen with		
Kreis value	204	0 57**
Stamm value with		
Kreis value	204	0 73**

** *Indicates 1% level of statistical significance*

alpha-dicarbonyl value and peroxide oxygen content were most closely associated. The Kreis values showed a logarithmic association with alpha-dicarbonyl and peroxide oxygen content The results suggest that formation of peroxides is more closely associated with alpha-dicarbonyl compounds, believed present in increasing quantities in rancid fat (16), than with epihydrin aldehyde, which may also be present (15), and which is supposedly responsible for the Kreis test

Discussion

Although most of the results of the chemical methods were highly associated with those of organoleptic rancidity, the peroxide oxygen and alpha-dicarbonyl measurements appeared to have more advantages than the others As peroxides are not thermostable, the peroxide oxygen content is usually altered by

processing techniques such as deodorizing or bleaching. Thus, substantial oxidation may have taken place, but the material may have only a small peroxide value. The measurement of the stable alpha-dicarbonyl compounds although less precise is considered a better method for the assessment of rancidity.

It is of interest to note the disappearance of fluorescent materials with the appearance of rancid odours at the end of the induction period. This indicates that fluorescence in lard may be linked with natural antioxidant substances, which are altered by oxidation.

Acknowledgments

References

1. CHAPMAN, R. A. and McFARLANE, W. D. Can. J. Research, B, 21 : 133-139. 1943.
2. COOK, W. H. and WHITE, W. H. Food Research, 4 : 433-440. 1939.
3. EVELYN, K. A. J. Biol. Chem. 115 : 63-75. 1936.
4. FELLENBERG, T. VON. Mitt. Lebensm. Hyg. 15 : 198-208. 1924.
5. FREHDEN, O. Mikrochim. Acta, 2 : 214-217. 1937.
6. FRENCH, R. B., OLCOTT, H. S., and MATTILL, H. A. Ind. Eng. Chem 27 : 724-728. 1935.
7. GRANT, G. A., MARSHALL, J. B., and LIPS, H J. In preparation.
8. KORPÁCZY, S. Z. Untersuch. Lebensm. 67 : 75-79. 1934.
9. KREJS, H. Chem. Ztg. 26 : 897. 1902.
10. LEA, C. H. Proc. Roy. Soc. London, B, 108 : 175-189. 1931.
11. LIPS, A., CHAPMAN, R. A., and McFARLANE, W. D. Oil & Soap, 20 : 240-243. 1943.
12. O'DANIEL, L. and PARSONS, L. B. Oil & Soap, 20 : 72-74. 1943.
13. PEARCE, J. A. Can. J. Research, F, 22 : 87-95. 1944.
14. PEARCE, J. A. and THISTLE, M. W. Can. J. Research, D, 20 : 276-282. 1942.
15. POWICK, W. C. J. Agr. Research, 26 : 323-362. 1923.
16. PRILL, E. A. Oil & Soap, 19 : 107-109. 1942.
17. ROSCHEN, H. L. and LEHMANN, W. K. Oil & Soap, 14 : 17-19. 1927.
18. STAMM, J. Analyst, 51 : 416-417. 1926.
19. RADLEY, J. A. and GRANT, J. Fluorescence analysis by ultraviolet light. Chapman and Hall, London. 1933.
20. WALTERS, W. P., MUERS, M. M., and ANDERSON, E. B. J. Soc. Chem. Ind. 57 : 53T-56T. 1938.
21. WHITE, W. H. Can. J. Research, D, 19 : 278-293. 1941.
22. YOUNG, C. A., VOGT, R. R., and NIEUWLAND, J. A. Ind. Eng. Chem., Anal. Ed. 8 : 198-199. 1936.

DRIED MILK POWDER

VI. THE EFFECT OF GAS- AND VACUUM-PACKING ON KEEPING QUALITY

By Jesse A. Pearce[2] and W. A. Bryce[3]

Abstract

Skim (1% fat) and whole (26, 28, and 30% fat) milk powders (2% moisture) from two plants were packed in air, carbon dioxide, nitrogen, 80% carbon dioxide and 20% nitrogen, 20% carbon dioxide and 80% nitrogen, and under vacuum, and stored for 12 months at 80° F. Quality was assessed by a tasting panel of 14 persons. Packing in an inert gas or under vacuum effected a general improvement in the quality of skim-milk powders. This was attributed to removal of volatile degradation products during the packing process and early storage. The storage life of whole milk powders was increased from a maximum of three months when packed in air to nine months when packed in inert gases or vacuum.

Introduction

Packing milk powders in nitrogen or carbon dioxide or in mixtures of both has become common commercial practice, but the effectiveness of these gases for preserving milk powders has been the subject of some controversy. The use of nitrogen only was reported to have no beneficial effect (14), but other investigations indicated that it provided protection (15), particularly if the oxygen concentration of the headspace gas was maintained at a low level (5, 8). The use of carbon dioxide has been variously reported as favourable (8, 15), without beneficial effect (14, 16), and harmful (7) to stored, dried, whole milk. Other reports indicated that mixtures of these two gases provided protection to stored milk powders (3, 6) and that packing under vacuum or partial vacuum had a beneficial effect on stored milk powders (7, 14, 15).

Studies on dehydrated egg-and-milk mixtures have shown that packing in carbon dioxide extends the storage life of this product (13). Carbon dioxide had a greater preservative effect on stored egg powder than nitrogen, which was in turn better than air (12).

The above results show that marked disagreements exist in the published investigations on the effect of gas-packing on the storage life of dried milk. It was, therefore, deemed advisable to compare the effect of packing in air, carbon dioxide, nitrogen, mixtures of carbon dioxide and nitrogen, and under vacuum, on milk powders of different fat levels from different sources. The present paper describes this investigation.

Materials and Methods

The materials used were those described in an earlier paper of this series (1) and consisted of powders of 1, 26, and 28% butterfat from one plant and powders of 1, 26, 28, and 30% butterfat from another. The powders were tempered to a moisture content of 2% by vacuum desiccation over phosphorus pentoxide and were packed in tinplate containers.

Occluded air (oxygen) may effect the storage life of gas-packed milk powder (3, 6, 8, 9). Therefore, in the packing technique used, an attempt was made to reduce the occluded gas to a minimum and to have the same amount in each sample. Using a previously described apparatus (2), the chamber containing the tins was evacuated to 1 mm. pressure, flooded with gas of the desired composition, evacuated as before, flooded again, and sealed. In vacuum packing, the tins were held under 1 mm. pressure for 15 min. and sealed at this pressure. The gases used were nitrogen, carbon dioxide, 80% carbon dioxide and 20% nitrogen, and 20% carbon dioxide and 80% nitrogen. Although it is believed that carbon dioxide may not be an "inert" gas when used to protect stored fat (7), the gas and vacuum-packs will be grouped under the heading "inert packs."

The foregoing samples and control samples with air as the headspace gas were stored at 80° F. and examined by palatability assessment initially and after 1, 3, 6, 9, and 12 months. To assess palatability, the samples were reconstituted as previously described (10), and sampled by 14 tasters. Scoring was done on a scale of 10 (the equivalent of excellent, fresh whole or skim-milk) to 0 (repulsive). A score of 4 is considered the point at which milk is no longer suitable for use as a milk drink. The reliability of the scoring by the taste panel has been estimated and palatability assessment was found to be more suitable than any of the chemical tests of milk powder quality (10). The desirability of using organoleptic tests as well as chemical tests on stored milk powders has been observed by others (4).

Results

The scores for the skim-milk powders (1% butterfat) at the 1 to 12 month samplings and scores for the whole milk powders (26, 28, and 30% butterfat) at the 1 to 9 month samplings were subjected to analysis of variance. The factors found to be significant are shown, in Fig. 1, by curves drawn through the mean palatability values for the various sampling times.

As noted previously, these skim-milk powders improved in quality during the first month of storage (1). Contrary to these earlier results (1), skim-milk powders from the two sources, when stored in an atmosphere of air, did not differ significantly in quality, but after 12 months' storage in inert atmospheres or in vacuum the powder from Plant 2 was considered half a palatability unit better than powder from Plant 1. Although no one method of obtaining an inert pack was significantly better than any other, after 12 months' storage, skim-milk powders in inert packs were significantly better than the air-packed material (about one palatability unit).

The air-packed whole milk powders deteriorated in a manner similar to that previously described (1); powders of 26 and 28% fat from Plant 2 had the poorest keeping quality; powder of 30% fat was better; and powders of 26 and 28% fat from Plant 1 had the best storage life. As observed in the previous study (1), there was no significant difference between powders of 26 and 28% fat from either plant.
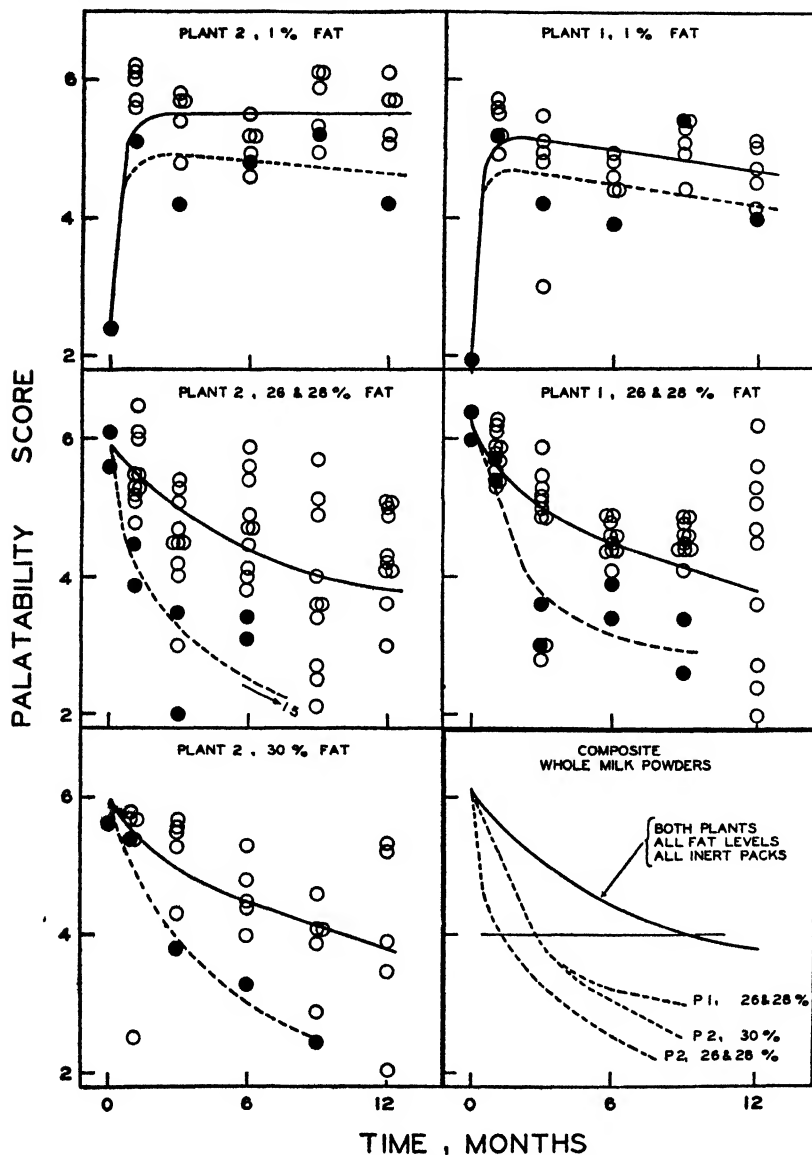

FIG. 1.   The effect of gas- and vacuum-packing on skim and whole milk powders stored at 80° F.

————————○———————— gas- or vacuum-packed material.
- - - - - - - - - ● - - - - - - - - - air-packed material.

The average loss in quality was the same in all whole milk powders packed in an inert atmosphere or under vacuum. No one method of obtaining an inert pack lengthened storage life significantly more than any other. However, the use of inert packs extended the average life of whole milk powders, normally one to three months, to nine months.

Discussion

The consistently higher palatability scores for the skim-milk powders packed in inert atmospheres and under vacuum support a previous suggestion that degradation products, responsible for low quality, are dissipated during the first few weeks after repacking (11) Beneficial effects of inert packing were evident after one month, and this superiority was maintained throughout the storage period. It is possible that subjecting these powders to low pressures during repacking removed quantities of volatile degradation products in excess of those dissipated when repacking in air only.

The variation in the rate of quality deterioration of the air-packed whole milk powders shows the effect on fat stability of processing practice in the different plants. Gas- or vacuum-packing reduced this difference in fat stability. While dissipation of degradation products may have been partly responsible for this improved storage life of gas- or vacuum-packed whole milk powders, the increasing differences in palatability, as storage progressed, supports previous evidence (5, 8) that reduction in the oxygen content of the gas surrounding the powder particles minimizes deterioration.

Deteriorative changes in whole milk powders packed in inert atmospheres approached those of air-packed skim-milk powder. This and other factors (11) indicated that solids-not-fat also play an important role in the deterioration of whole milk powders

A wide variation was observed in the scores applied to the powders packed in inert gases or under vacuum. This variation includes the effect of a number of factors: source of powder, fat level, method of obtaining an inert pack, variability in the milk powder samples, and variability of taster scores. These results may explain the observed differences in effectiveness of methods of gas-packing. Examination of the scatter shows that some samples in inert packs were judged to be of lower quality than air-packed samples, while others were considered to be about the same quality as the air-packed samples. Therefore, it is possible that samples in inert packs, when examined by one investigator, might appear no better than air-packed samples, while another examination would show inert packing to have a beneficial effect. The results presented here show that the average effect of inert packing is a prolongation of the storage life of dried milk powder.

References

1. BRYCE, W. A. and PEARCE, J. A. Can. J. Research, F, 24 : 61-69. 1946.
2. BRYCE, W. A. and TESSIER, H. Can. J. Research, F, 23 : 304-305. 1945.
3. COULTER, S. T. and JENNESS, R. Food Industries, 18 (3) : 96-99; 212-216. 1946.

4. GREENBANK, G. R., WRIGHT, P. A., DEYSHER, E. F., and HOLM, G. E. J. Dairy Sci. 29 : 55-61. 1946.

5. HETRICK, J. H. and TRACY, P. H. J. Dairy Sci. 28 : 687-700. 1945.

6. HOLM, G. E. and GREENBANK, G. R. Proc. World's Dairy Congr. 2 : 1253-1265. 1923.

7. HOLM, G. E., WRIGHT, P. A., and GREENBANK, G. R. J. Dairy Sci. 10 : 33-40. 1927.

8. LEA, C. H., MORAN, T., and SMITH, J. A. B. J. Dairy Research, 13 : 162-215. 1943.

9. PALMER, L. S. and DAHLE, C. D. J. Dairy Sci. 5 : 240-245. 1922.

10. PEARCE, J. A. Can. J Research, F, 23 : 177-184. 1945

11. PEARCE, J. A. and BRYCE, W. A. Can. J. Research, F, 23 : 334-339. 1945.

12. PEARCE, J. A., REID, M., and COOK, W. H. Can J Research, F, 24 : 39-46 1946.

13. PEARCE, J. A., WHITTAKER, J., TESSIER, H., and BRYCE, W A. Can. J. Research, F, 24 : 70-76. 1946.

14. SCHATZEL and CLAUSEN. Molkerei-Ztg. 53 : 1532-1534. 1939. *Abstracted in* Chem. Abstracts, 34 : 4169. 1940.

15. SUPPLEE, G. C. Proc. World's Dairy Congr. 2 : 1248-1253 1923.

16. WOODCOCK, A. H. Can. J. Research, F, 23 : 117-122. 1945.

DRIED WHOLE EGG POWDER

XXII. SOME FACTORS AFFECTING THE PRODUCTION AND INITIAL QUALITY OF DRIED SUGAR-AND-EGG MIXTURES

By Jesse A. Pearce, J. Brooks, and H. Tessier

DRIED WHOLE EGG POWDER

XXII. SOME FACTORS AFFECTING THE PRODUCTION AND INITIAL QUALITY OF DRIED SUGAR-AND-EGG MIXTURES[1]

By Jesse A. Pearce[2], J. Brooks[3], and H. Tessier[4]

Abstract

Sugar–egg powder was produced under a variety of conditions in a laboratory spray drier and in two commercial driers. A product prepared at inlet temperatures below 270° F. and outlet temperatures below 150° F. was the most suitable for baking purposes and was generally the best when assessed by measurements of fluorescence, potassium chloride value, and pH. Powder of particle size small enough to pass an 80 mesh screen (U.S. Bureau of Standards) appeared to have better baking properties than coarser material. Trials with nozzles of various sizes indicated that the best product was prepared using small nozzles. Sucrose syrup or solid sucrose, with fresh or frozen egg, all produced powders of similar initial quality.

Introduction

The addition of sucrose sugar to liquid egg before drying is known to provide protection during heat treatment (2) and storage of the dried product (1, 3). The product is much more suitable than plain egg powder, for use in baked goods (2). However, no information was available about the best conditions for producing this material. Since it is expected that Canada will produce about 20¼ million pounds of dried sugar–egg powder during 1946, it was believed desirable to examine certain factors in the processing procedure that may affect the quality.

Materials and Methods

The liquid from fresh, Grade *A*, shell eggs, except as noted in Fig. 1, was used in operations on the laboratory cone-type drier (11), while liquid from frozen egg was used for all work on two commercial cone-type driers, except as noted in Table II.

Rate of production at specific drying conditions for some of the work done in the commercial plants is given in Table I. The effect of air temperature and nozzle diameter on the rate of powder production can be estimated from Table I and the data in the other tables and Fig. 1.

The quality of the powder was determined by measurement of the moisture content (9), fluorescence value (5), potassium chloride value (9), pH (4), baking volume (6), foaming volume (6), and foam stability. In addition,

TABLE I

DRYING CONDITIONS AND RATE OF SUGAR–EGG PRODUCTION FOR SOME OF THE WORK DONE IN THE COMMERCIAL PLANTS

Plant	Inlet temperature, ° F.	Outlet temperature, ° F.	Nozzle diameter, in	Pump pressure, p s i	Production, lb./hr.
1	285	155	0 0635	4300	700
2*	240	155	0 0700	4400	700

* *Uses preheater on egg just before it goes to spray nozzle in drier.*

some samples were subjected to a sieve analysis to obtain fractions of different particle sizes. The procedures for determining baking volume and foaming volume were unsatisfactory and were modified as noted below.

To prepare sponge cakes for the determination of baking volume, all the materials used were brought to a temperature of 80° F. and all mixing was done in a room at 80° F. and 65% relative humidity. The procedure was as follows.

Sugar–egg powder (9 gm.) was mixed, by hand, with 17 gm. of commercial, powdered sugar (sucrose) in the small bowl of a Mixmaster. A portion of a measured volume of 19 ml. of tap water was added and mixed into a paste with the sugar and powder. The remainder of the water was added and mixed with the paste until a homogeneous liquid was obtained. The beaters of the Mixmaster were then lowered into the bowl so they just touched the bottom and were allowed to whip the mixture for 10 min. while operating at No. 10 speed. After five minutes' beating, the bowl was turned by hand through 90°, but no other motion of the bowl was permitted. Small portions of a 20 gm. quantity of a standardized super-cake flour were sprinkled over the surface and each portion was carefully mixed in with a rubber spatula in such a manner that the foam was disturbed as little as possible. The batter was then carefully scraped from the beaters and bowl and transferred to an ungreased pan, which was immediately inserted into an oven (400° F.) and baked for 15 min. After baking, the sponge and pan were inverted and allowed to stand in the conditioned room overnight The next day, the volume of the cake was measured. The standard deviation of this volume-measuring technique was 2 4 ml.

It was observed that two different technicians produced cakes with an average difference in volume of 12 ml., therefore, most of the baking was done by one person only. For this person, three cakes were necessary to show a significant difference of 10 ml. in the baking volume of the powders. Therefore, all values shown are the average of volume measurements on three cakes. No significant day-to-day differences in cake volume were observed.

To determine foaming volume the requirements for conditioning and mixing the materials were the same as for the baking test. Otherwise, the

quantities of material and the procedure described elsewhere were used (2). Foam stability was evaluated by inverting the graduate cylinder, in which foaming volume was measured. The time required for the foam to begin dripping from the cylinder was determined
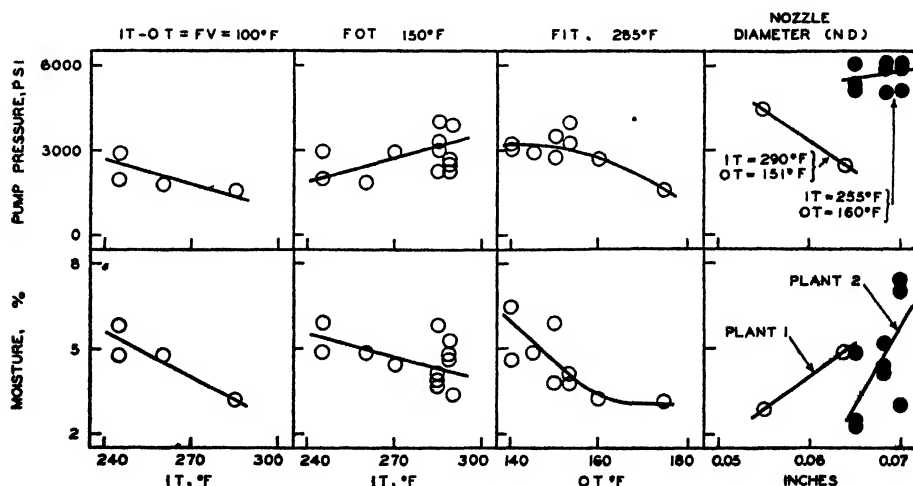
LABORATORY   DRIER
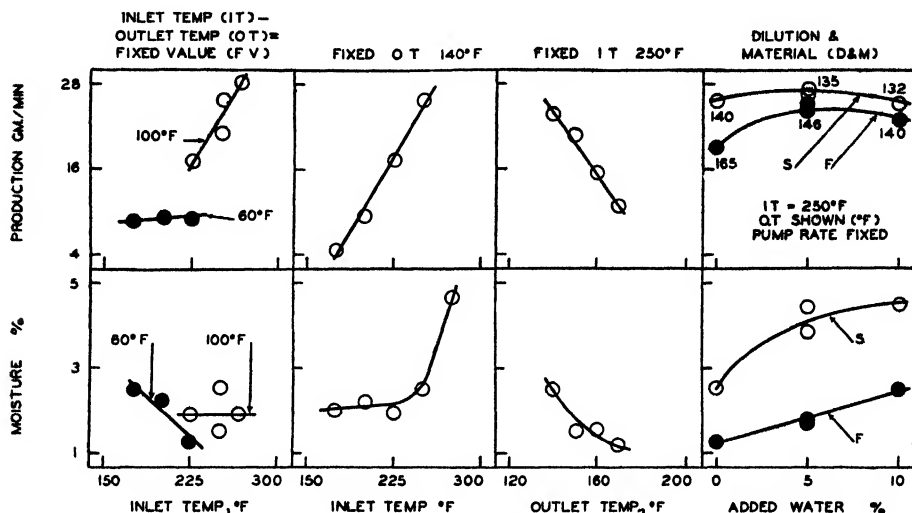
COMMERCIAL   DRIERS

FIG 1   *The effect of drying conditions on the moisture content and rate of production (shown as pump pressures in commercial driers) of sugar–egg powder*

Laboratory drier.   O, powder from fresh shell eggs (S),   ●, powder from frozen liquid eggs (F).

Commercial drier   O, operations at Plant 1,   ●, operations at Plant 2.

Particle size separations were done using screens of 16, 35, 65, 100, 200, and 325 mesh (U.S. Bureau of Standards). Previous examination indicated that one hour on a Ro-tap shaker was the most desirable sieving time, and this sieving period was used throughout. However, it has since become apparent that even this time did not provide complete separation of particles of different size (8).

Results

Laboratory Drier

The use of low drying temperatures reduced the rate of production and resulted in increased moisture content in the powder (Fig. 1); this corroborated the results of a previous study on plain egg powder (10). However, powder produced at an inlet temperature of 280° F. and an outlet temperature of 140° F. had an unexpectedly high moisture content. This may be attributable to the low volume of air passing through this drier and the high liquid flow rates necessary to maintain the low outlet temperature. Under these conditions, adequate removal of the water vapour was not possible.

As with plain egg powder (10), the present work showed that the best product, as assessed by all quality measures, was produced at the lowest temperatures (Fig. 2). One anomalous result was noted in the studies using fixed outlet temperatures. Materials produced at an outlet temperature of 150° F. had lower fluorescence values than material produced at 140° or 160° F. These products also had higher foaming volumes than material produced at 140° F. In general, the results indicated that, for this drier, good quality sugar–egg powder could be produced at an inlet temperature of 270° F. and that outlet temperatures of about 140° to 150° F. were satisfactory.

For a fixed pump rate a smaller amount of powder was produced from frozen melange than from fresh liquid egg (Fig: 1). This was attributable to the greater viscosity characteristic of stored, frozen, liquid egg. (It is possible that this high viscosity might be reduced by the addition of sugar to the liquid egg before freezing.) The moisture content was lower owing to the higher outlet temperatures associated with the lower throughput. However, by appropriate dilution, it was possible to prepare material from frozen melange that was similar in quality to that prepared from fresh eggs. As might be expected, at a fixed pump rate, dilution reduced the outlet temperature, with a corresponding increase in powder quality and moisture content.

Several additional factors were examined using the laboratory drier (Table II). These results showed that no significant difference resulted from the use of solid sugar or sucrose syrup, or from the sugars currently available in Canada and likely to be used by the various producers. Rapid beating of the sugar and liquid egg before drying increased the fluorescence value and decreased the potassium chloride value although it had no significant effect on baking quality.
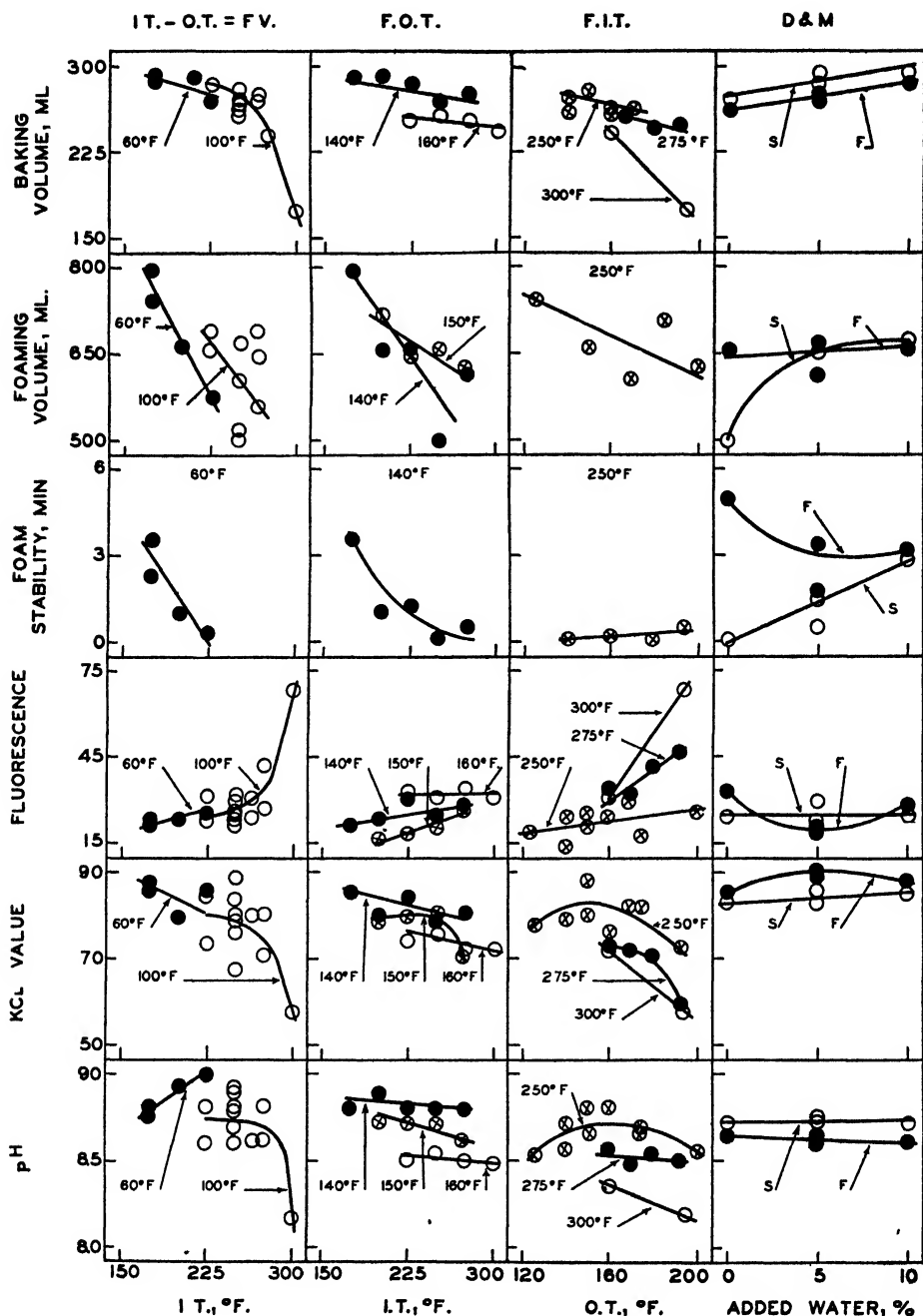
FIG 2. The effect of drying conditions on the quality of sugar–egg powder produced on laboratory drier (for abbreviations, see Fig. 1): ○, powder from fresh shell eggs (S), ●, powder from frozen liquid eggs (F).   The average baking volume for sponges prepared from shell eggs was 286 ml.

TABLE II

ErFECT OF MISCELLANFOUS ITEMS ON POWDFR PRODUCTION AND POWDER QUALITY—LABORA1ORY DRIER

Item	Number of trials	Inlet temp °F	Outlet temp °F	Powder production gm /min	Powder quality						
					Mois ture %	Baking volume ml	Foaming volume ml	Foam stabil ity min	Fluor escence value	Potas sium chloride value	pH
Sugar vs syrup (about 55% sucrose)											
Set 1											
Sugar	9	250	150	18 8	2 8	—	662	—	22 1	76 7	8 6
Syrup	9	250	150	14 2	4 0	—	682	—	21 1	78 6	8 6
Set 2											
Sugar	3	225	160	—	—	273	—	—	34 8	78 8	8 7
Syrup	3	225	160	—	—	265	—	—	34 6	77 1	8 5
Effect of sugar from different areas											
Alta (beet)	3	250	140	—	—	284	670	2 5	17 7	81 2	8 8
Man (beet)	3	250	140	—	—	286	687	2 5	18 3	83 5	8 8
Ont (cane)	3	250	140	—	—	286	710	4 0	18 2	81 1	8 8
Effect of rate of stirring egg and sugar before drying											
Fast (causes foaming)	2	250	140	25 8	2 78	280	633	1 5	26 1	84 8	8 8
Slow (no foaming)	2	250	140	25 8	3 05	276	656	1 9	21 8	88 0	8 6

Commercial Driers

For the commercial driers, as for the laboratory drier, it was apparent that reduction in the inlet temperature, with a fixed outlet temperature, decreased production (estimated from pump pressure changes noted in Fig 1) and resulted in a product with increased moisture content Reducing the diameter of the spray nozzle lowered the moisture content, but did not affect production if the pump pressure was increased

By all quality criteria, except foam stability and pH, the best product was produced at the lowest drying temperatures (Fig 3) The foam stability measurement gave irregular results but this measurement was believed to be of less importance than baking volume No explanation can be offered for the exceptionally high pH values observed for material produced at inlet temperatures higher than 285° F. for Plant 1 and 280° F for Plant 2 In general, inlet temperatures of 270° F. and lower were most satisfactory. For Plant 1, an outlet temperature of 150° F. or lower was most desirable. Because of
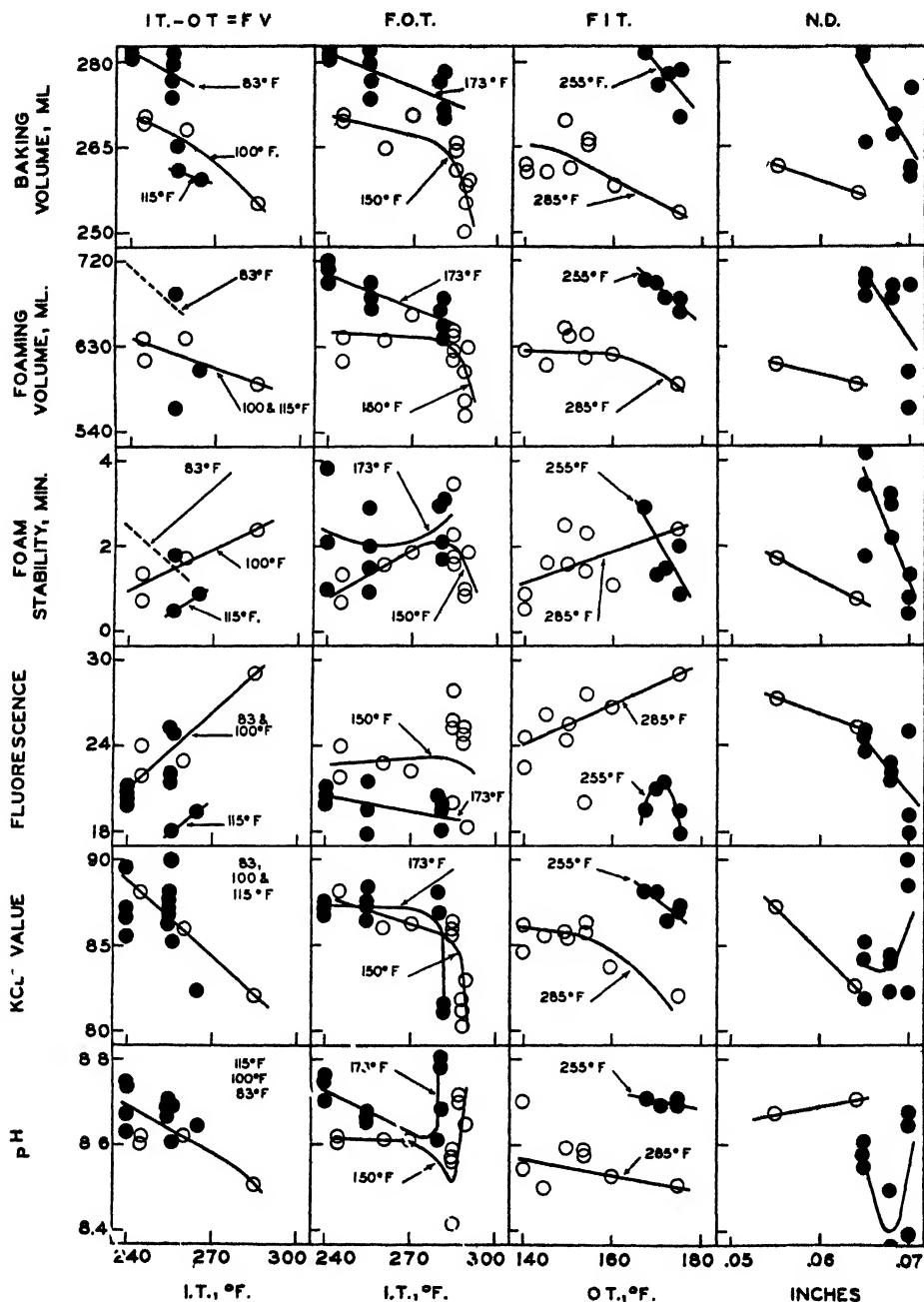
FIG. 3.   *The effect of drying conditions on the quality of sugar–egg powder produced on commercial driers (for abbreviations, see Fig. 1): ○, operations at Plant 1; ●, operations at Plant 2; dotted lines, trials at Plant 2, points omitted to avoid confusion.   All studies shown in the first three columns done at nozzle diameters of 0 064 in. and 0 070 in Plants 1 and 2, respectively.   The average baking volume for sponges prepared from shell eggs was 286 ml.*

the limited range studied it was difficult to evaluate the most desirable outlet temperatures for Plant 2, but, again, the indications were that the lowest temperatures were the most desirable.

The size of the spray nozzle used was important. Results from both plants showed that the smaller the nozzle diameter the better the baking quality of the product and the lower the moisture content of the powder, but the fluorescence values were slightly increased. Nevertheless, the powders produced would meet the requirements of the tentative specification for Grade A sugar–egg powder (7). Further trials in Plant 2, using a multiple nozzle (three openings, 0 055 in.), gave products with baking volumes of 289 and 291 ml.

Baking tests were also done on fractions sieved from the various powders (Table III). These results supported the foregoing evidence and indicated that, for best baking quality, the powder should be fine enough to pass an 80 mesh screen.

TABLE III

THE EFFECT OF PARTICLE SIZE ON THE BAKING QUALITY OF SUGAR–EGG POWDER

Baking volume of whole sample, ml	Baking volume (ml) of sieved portions falling between the following sieve sizes (U S. Bureau of Standards).					
	16-35	35-65	65-80	80-100	100-200	200-325
283	—	—	262	273	279	—
282	—	–	—	—	276	279
269	—	274	282	—	272	—
266	—	267	—	264	272	—
266	—	275	265	—	257	—
265	—	259	254	—	265	—
265	—	228	250	—	252	254
264	—	268	272	—	271	—
259	—	268	261	275	269	—
259	248	254	256	—	257	—
Average	248	262	263	271	263	266

Several additional factors were examined (Table IV). The increased temperature difference necessary when producing powder in wet weather caused a slight increase in fluorescence value and a slight decrease in potassium chloride and foaming volume values. The temperature of the powder at the time of packing had no significant effect on the baking quality of the product. Differences in powders produced from diluted liquid egg (fresh, shell), mixtures of frozen and shell egg liquids, and frozen egg were not significant. Powders of similar quality were produced by the addition of sugar in either solid or liquid form.

Discussion

The results obtained using either the laboratory or commercial driers showed that sugar–egg powder with excellent baking properties can be produced at inlet temperatures of 270° F. or lower and at outlet temperatures

TABLE IV

EFFECT OF MISCELLANEOUS ITEMS ON POWDER PRODUCTION AND POWDER
QUALITY—COMMERCIAL DRIERS

Item	Number of trials	Inlet temp., ° F.	Outlet temp., ° F.	Powder production, gm./min	Powder quality						
					Moisture, %	Baking volume, ml.	Foaming volume, ml.	Foam stability, min.	Fluorescence value	Potassium chloride value	pH
Dry weather	9	285	255	3244	3 92	262	632	2 2	22 7	86 2	8.6
Wet weather	8	290	250	3098	4 67	260	593	1.2	24 1	84 3	8.6

Powder packaged at average temperature of 78° F.

Plant 1	2	289	152	3170	4 05	254	610	1 4	21 7	83 6	8 7
Plant 2	1	255	162	6000	4 12	267	695	3 0	21 6	84 1	8 4
Average					4 07	258	638	1 9	21.7	83 8	8 6

Powder packaged at average temperature of 106° F.

Plant 1	2	288	151	2400	5 23	256	564	1 0	24 5	82 5	8 7
Plant 2	1	255	162	6000	4 33	270	685	3 5	22 9	84 8	8 2
Average					4 93	261	604	1 8	24 0	83 3	8 5

Frozen vs. fresh shell liquid, and dilution

Shell (undiluted)	3	285	152	3300	3 22	260	627	2 0	21 4	86 8	8 6
Shell plus 7% water	1	285	154	3300	3 81	260	617	2 5	25 8	87 2	8 6
1 part shell and 3 parts frozen	1	285	153	3000	4 01	264	628	3 5	25 5	86 2	8 4
Frozen plus 7% water	4	285	154	3680	3 86	266	616	2 0	23 0	86 8	8 6

Sugar vs. syrup (about 55% sucrose)

Plant 1											
Sugar	3	285	154	3350	4 02	265	640	2 3	20 2	86 8	8 6
Syrup	3	285	155	3300	3 76	257	611	2 2	22.7	86 0	8 6
Plant 2											
Sugar	1	257	155	6000	4 94	266	685	1 8	24 9	85 2	8 6
Syrup	1	255	160	5300	2 52	283	688	3 4	25 0	83.8	8.6

of 150° F. or lower. These conditions permitted fairly rapid production and, when small nozzles were used, produced powder with less than 3.0% moisture (2). It is also of interest to note that in the commercial trials the plant using a preheater for the liquid egg produced powder with better baking quality.

The present results showed that no improvement in initial baking quality resulted from cooling the product before packaging. Heat treatment studies have shown that rapid cooling of the product on removal from the drier is an essential (2).

Although the average value obtained for the baking volume of cakes from shell eggs of varying quality was 286 ml. (range 266 to 305 ml.), powder prepared on the laboratory drier produced cakes with baking volumes as high as 300 ml. This was attributed in part to the use of fresh shell eggs in this drier, but the major factor believed responsible was the use of an extremely small nozzle (0.025 in.).

While this study showed little difference in the initial quality of products prepared using solid sugar or sucrose syrup and using fresh eggs and frozen melange, it has been observed elsewhere that the use of syrup and the use of frozen melange resulted in products that were less stable when stored (2). In addition, the use of syrup necessitates the removal of a greater quantity of water, thereby increasing the cost of production.

Acknowledgments

References

1. BROOKS, J. and HAWTHORNE, J R. J. Soc. Chem. Ind. 62 : 165-167. 1943.
2. HAY, R. L. and PEARCE, J. A. Can. J. Research, F, 24 : 168-182. 1946.
3. HAY, R. L. and PEARCE, J A. Can. J. Research, F, 24 : 430-436. 1946.
4. NATIONAL RESEARCH COUNCIL, CANADA, and SCIENCE SERVICE OF THE DEPARTMENT OF AGRICULTURE, CANADA The official methods used for assessing quality in Canadian dried whole egg powders. Ottawa, 28 Feb. 1944.
5. PEARCE, J. A , THISTLE, M. W , and REID, M. Can. J. Research, D, 21 : 341-347. 1943.
6. REID, M. and PEARCE, J A Can J. Research, F, 23 : 239-242. 1945.
7. SPECIAL PRODUCTS BOARD OF THE DEPT OF AGRICULTURE, CANADA. Tentative requirements for sugar–dried whole egg powder. Ottawa. 1946.
8. TESSIER, H., MARIER, J. R , and PEARCE, J. A. In preparation.
9. THISTLE, M. W., PEARCE, J. A , and GIBBONS, N. E. Can. J. Research, D, 21 : 1-7. 1943.
10. WOODCOCK, A. H. and REID, M. Can. J. Research, D, 21 : 389-393. 1943.
11. WOODCOCK, A. H. and TESSIER, H. Can. J. Research, A, 21 : 75-78. 1943.

DRIED WHOLE EGG POWDER

XXIII. THE EFFECT OF MOISTURE CONTENT AND METHOD OF PACKING ON THE STORAGE LIFE OF DRIED SUGAR-EGG MIXTURES

BY R. L. HAY AND JESSE A. PEARCE

DRIED WHOLE EGG POWDER

XXIII. THE EFFECT OF MOISTURE CONTENT AND METHOD OF PACKING ON THE STORAGE LIFE OF DRIED SUGAR-EGG MIXTURES[1]

By R. L. Hay[2] and Jesse A. Pearce[2]

Abstract

Dried sugar–egg powders, obtained from a commercial Canadian source, were adjusted to 1.4, 2.8, and 3.2% moisture and stored at 40°, 80°, and 120° F. from 1 to 52 weeks. Quality of the powder was assessed by measurement of fluorescence, potassium chloride value, pH, and foaming volume. The rate of deterioration increased with an increase in moisture content at 80° and 120° F. The effect of moisture content on fluorescence and potassium chloride values was negligible at 40° F., but high moisture in powders stored at this temperature accelerated the development of acidity and the loss in baking quality as assessed by foaming volume

Packing in carbon dioxide, nitrogen, and *in vacuo* had a slight beneficial effect on dried sugar–egg powder.

Introduction

Lowering the moisture and volatile content of plain egg powder to 2% has been found to exert a definite beneficial effect on storage life (10). Moisture levels of less than 1% improved the keeping qualities of dried albumen and whole egg but not of dried yolk (7). In a recent investigation, sugar–egg powder tempered to a 1.4% moisture level was considerably better than a similar powder with a 2.8% moisture content, when held at elevated temperatures (1). Packing in carbon dioxide had a definite preservative action on stored plain egg powder but packing in nitrogen and under vacuum had no beneficial effect (9, 11).

The present paper deals with the effects of low moisture content and of packing with carbon dioxide, nitrogen, and *in vacuo* (inert packs) on the keeping quality of sugar–egg powder stored for one year.

Materials and Methods

The sugar–egg powder (33% sugar, dry basis) used in this investigation was similar to that described in an earlier paper (1) and was adjusted to moisture levels of 1.4, 2.8, and 3.2%. Samples at all moisture levels were sealed in an atmosphere of air: samples at 2.8% moisture were also sealed in carbon dioxide, nitrogen, and under vacuum. All were examined after storage for one, two, and four weeks at 120° F.; after 1, 2, 4, 8, 16, and 32 weeks at 80° F.; and after 16, 32, and 52 weeks at 40° F. The quality of the egg powder was assessed by measurement of fluorescence (4), potassium chloride value (8), pH (8), and foaming volume (1, 5).

During this investigation, it was observed that the foaming volume test was not entirely satisfactory and, as a result, a baking test has been substituted in other work in these laboratories (2). While foaming volume has proved inferior to the baking test, the former can be utilized to evaluate the baking quality of sugar–egg powder, when comparing powders from the same source.

Results

The results are presented in Figs. 1 to 4. It should be noted that the time intervals are shown as a geometrical progression, to permit graphical comparison of changes at high and low storage temperatures.
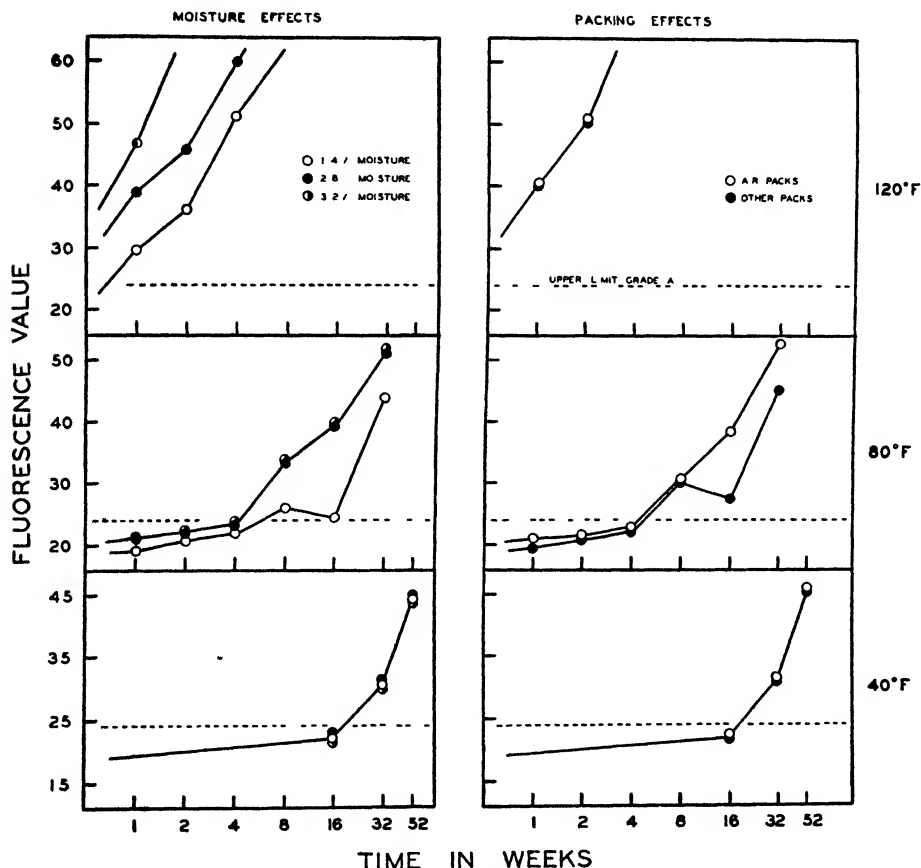
FIG. 1   Effect of moisture contents of 1 4 to 3 2% and method of packing on the fluorescence values of dried sugar–egg powders.

The Effect of Moisture Content

An increase in moisture content in the powder resulted in accelerated fluorescence development at 80° and 120° F. but had no measurable effect on the fluorescence of powders stored at 40° F. (Fig. 1). At 40° F. fluorescence

changes in the powders at all three moisture levels were negligible during the first 16 weeks, but fluorescence increased considerably and at equal rates for all moisture levels during the subsequent portion of the storage period. At 80° F. the fluorescence values of all powders increased slowly and at equal rates during the first four weeks, but subsequent changes in the 2.8 and 3.2% powders were much more rapid than in the 1.4% egg powder. At this temperature, reducing the moisture content from 2.8 to 1.4% appeared to
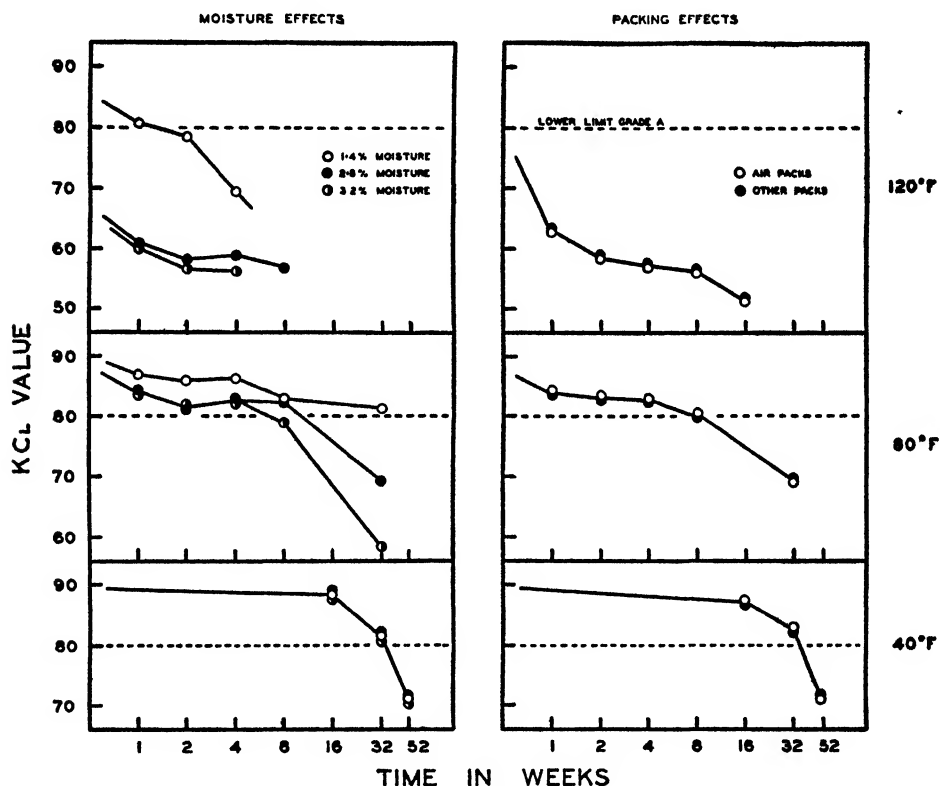
FIG. 2. *Effect of moisture contents of 1.4 to 3 2% and method of packing on the potassium chloride values of dried sugar–egg powders.*

increase the storage life of sugar–egg powder by about 12 weeks. The difference in behaviour between the several moisture levels was most marked at 120° F., the 1.4% powder remaining at the lowest fluorescence level during the entire storage period.

The behaviour of the potassium chloride values in this study (Fig. 2) agreed with and supported the results noted above for the fluorescence test. Lowering the moisture content from 3.2 to 1.4% did not appear to prolong the storage life of sugar–egg powder when stored at 40° F. for one year. However, at both 80° and 120° F. the beneficial effects of reduction in moisture content from 3.2 to 1.4% were quite marked. At 80° F. loss in quality of the 1.4%

powder was comparatively small.   The 3.2% samples deteriorated at approximately the same rate as those containing 2.8% moisture during the first four weeks of storage at 80° F., but showed a more rapid loss in solubility during the remaining portion of the storage period.   Changes in potassium chloride value were rapid in all powders stored at 120° F., the most marked occurring in the 2.8 and 3.2% powders during the first week.
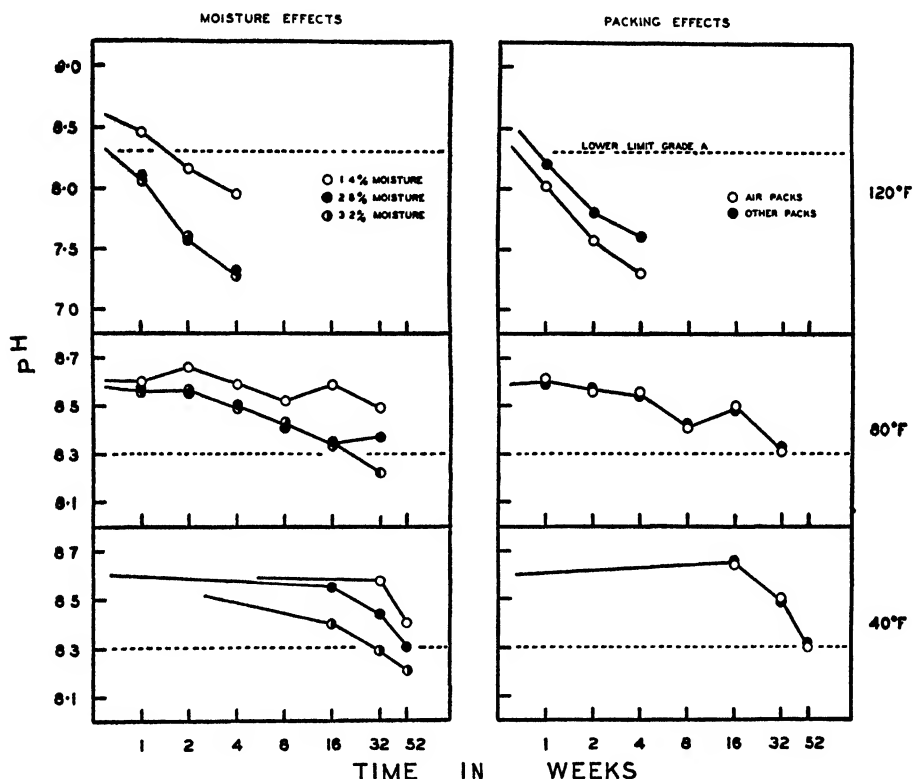

FIG. 3.   *Effect of moisture contents of 1 4 to 3 2% and method of packing on the pH values of dried sugar–egg powders*

The pH measurements (Fig. 3) gave further evidence that very low moisture contents are advantageous.   At all temperatures studied, powders containing 1.4% moisture maintained a higher pH level than either the 2.8 or 3.2% powders.   There was little difference between the 2.8 and 3.2% powders at the higher temperatures, but, unlike the other tests, this test showed that reduced moisture content prolonged storage life at 40° F.

Notwithstanding the irregularities shown in Fig. 4, the foaming volume measurements supported the desirability of maintaining a low moisture content in stored sugar–egg powders.   During a previous study (1), it was noted that sugar–egg powder with a high moisture content had a higher foaming volume after short storage periods than low moisture powder.   However,

after the powders had been stored for some time the foaming volume of the
high moisture powder decreased below that of the low moisture powder. In
the present study (Fig. 4) a similar effect was evident during storage at 40° F.

Effect of Methods of Packing

Of the powders stored at 40° F. for 52 weeks there was no evidence by any
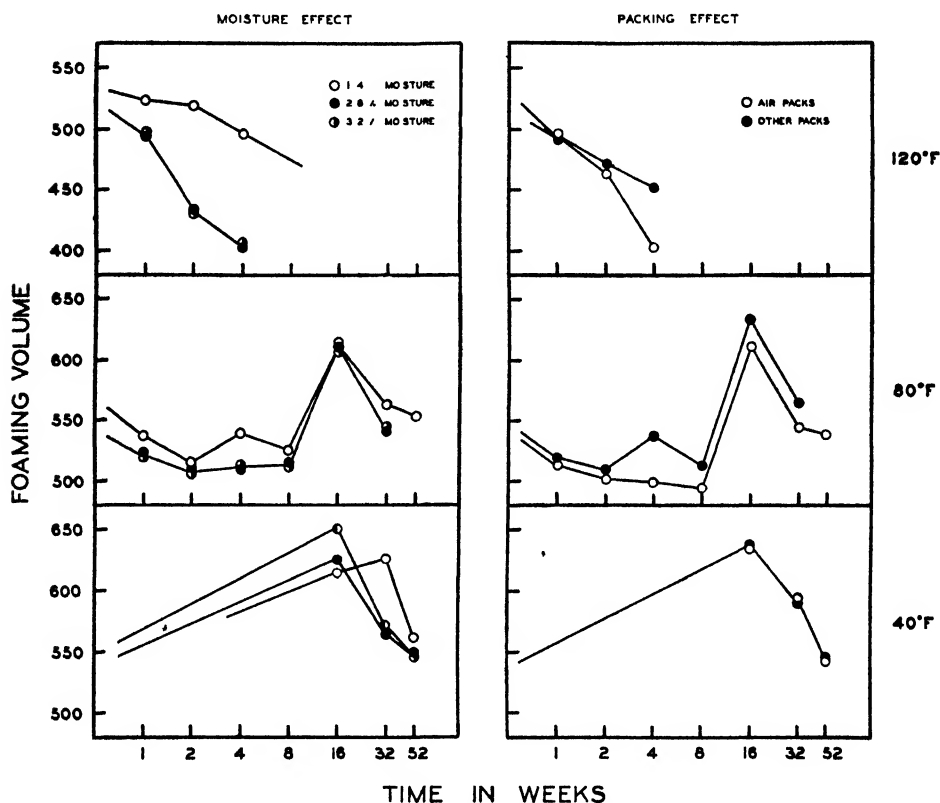test to show that gas-packing had a preservative effect on the quality of

FIG 4   *Effect of moisture contents of 1 4 to 3 2% and method of packing on the foaming
volumes of dried sugar–egg powders*

sugar–egg. At 80° F. the fluorescence test (Fig. 1) and the foaming volume
test (Fig 4) showed that the inert packs exerted some protective action during
storage. However, pH (Fig. 3) and potassium chloride values (Fig. 2) were
not affected by inert packing. Only pH and foaming volume measurements
showed a beneficial effect from the use of inert packs on powders stored at
120° F. Although inert packs appeared to retard quality deterioration
slightly, there was no evidence to show that any one was more effective than
the other two. The limited protection provided by this treatment does not
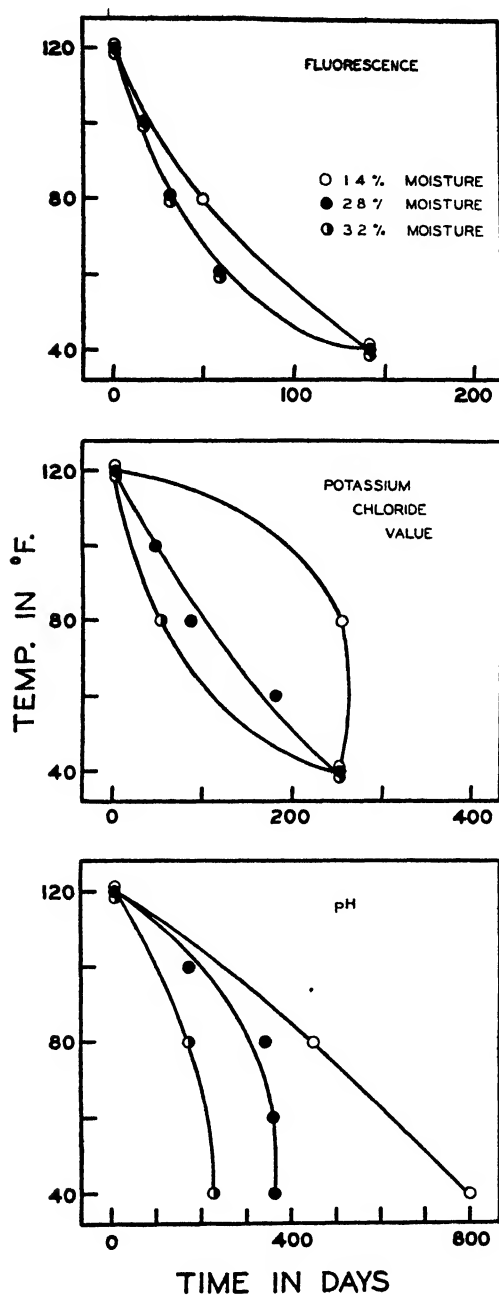seem to warrant its use in current commercial practice.

FIG 5. *Effect of moisture contents of 1 4 to 3 2% on the time required for sugar-egg powder to change from A to B quality   Values plotted at 40°, 80°, and 120° F were calculated from data in Figs 1, 2, and 3, those at 60° F. were from the present study but not shown otherwise; those at 100° F. from a previous study (1)*

Discussion

The importance of moisture content in these powders can be assessed by considering the length of time required for them to change from *A* quality to *B* quality (shown by dotted lines in Figs. 1, 2 and 3 and summarized in Fig. 5) according to the tentative specifications for sugar–egg powder produced in Canada (6). Reducing the moisture content from 2 8 to 1.4% appeared to have little effect on the fluorescence and potassium chloride values of powder stored at 40° F.; perhaps, at this temperature, the protective effect of added sugar (1) was sufficient to mask any advantage gained from a very low moisture content. However, the pH changes indicated that the life of Grade *A* egg powder stored at 40° F might be prolonged for more than one year when the moisture was reduced from 2 8 to 1 4%. Unless sugar–egg powder can be kept at temperatures of about 40° F. it seems advisable from these results to prepare powders with moisture contents below 2 8%, which are believed to be commercially feasible (2)

A previous investigation with plain egg powder showed that only carbon dioxide had a beneficial effect (11). The presence of added sugar in the egg powder used in this study apparently retards the reaction that contributes most to egg powder deterioration (probably a sugar–protein reaction (3)) and permits oxidation reactions to become more important. Hence any protective effect was common to all methods of obtaining an inert pack

Acknowledgment

References

1. HAY, R L. and PEARCE, J A   Can J Research, F, 24 168 182   1946
2. PEARCE, J. A , BROOKS, J , and TESSIER, H   Can J Research, F, 24 420–429   1946.
3. PEARCE, J A and BRYCE, W A   Proc Inst Food Tech   In press
4 PEARCE J A , THISTLE, M W , and REID, M   Can J Research, D, 21 341-347   1943.
5 REID, M and PEARCE, J A   Can J Research, F, 23 239 242   1945
6 SPECIAL PRODUCTS BOARD, DEPARTMENT OF AGRICULTURE, CANADA   Tentative requirements for sugar-dried whole egg powder   Ottawa   1946.
7. STEWART, G. F , BEST, L R , and LOWE, B   Proc Inst Food Tech 77-89   1943
8. THISTLE, M. W., PEARCE J A , and GIBBONS, N E   Can J. Research, D, 21 : 1-7. 1943
9 THISTLE, M. W , WHITE, W H , REID M , and WOODCOCK, A H   Can J. Research, F, 22 80-86   1944
10 WHITE, W H and THISTLE, M W   Can. J Research, D, 21 · 211 222   1943.
11. WHITE, W. H , THISTLE, M. W., and REID, M   Can J. Research, D, 21 : 271-276.   1943.

A FLUORESCENCE METHOD FOR ASSESSING THE KEEPING QUALITY OF BUTTER[1]

By G. A. Grant[2] and W. Harold White[3]

Abstract

The fluorescence values of serum from salted butter were affected by separation temperature, dilution, nature and pH of the diluent, and the stability of the diluted serum. A satisfactory procedure was as follows The serum was separated by placing 125 gm. of butter in centrifuge bottles and heating to 45° C. in a boiling water-bath, centrifuging at 1700 r p m , and siphoning off the fat. Two millilitres of the serum was diluted to 50 ml with 10% sodium acetate, the pH adjusted to 5–6 and the fluorescence determined immediately in a Coleman photofluorometer using a filter that transmitted light in the region of 365 mμ. This procedure gave fluorescence values that were correlated with flavour score ($r = -.84$) on salted butter stored at 32 2° C. (90° F)

Introduction

Numerous objective methods for assessing the quality of butter have been investigated without marked success. These include measurements of the aldehyde (9), peroxide (7), and free fatty acid (2) contents of the fat; and titratable acidity, hydrogen ion concentration, and amino nitrogen content of the serum (2). The measurement of fluorescence has been applied to a variety of foodstuffs (3, 4, 5) to assess the changes induced by storage. Concomitant organoleptic assessment of the products has shown marked relation between flavour deterioration and fluorescence values for some materials, e.g., powdered eggs, while for others the objective test is not at all indicative of flavour status. This is to be expected since, for the materials studied, fluorescence is an attribute of the salt extract of the defatted material and as such primarily reflects changes in the non-fat components. Increasing the moisture content of egg powder and ration biscuits (8, 3) brought about an increase in fluorescent material, and in the latter suppressed oxidative changes in the fat. These observations suggested that the serum separated from butter would contain fluorescing substances in amounts that increase as the butter deteriorates. This paper describes the factors affecting the measurement of fluorescence in fresh and spoiled salted butter, and demonstrates the changes in fluorescence value under accelerated storage conditions.

Procedure

Preliminary Trials

After several trials it was observed that serum and butter fat could be separated satisfactorily by heating 125 gm. of butter at 60° C. for five minutes

---

[1] *Manuscript received March 18, 1946.*

[2] *Laboratory Steward, Food Investigations.*

[3] *Formerly Biochemist, Food Investigations: now Chemist, Imperial Oil Company Limited, Sarnia, Ontario.*

---

and centrifuging for 10 min. at 1700 r.p.m. The fat was removed by siphoning and the clearest portion of serum pipetted into an Erlenmyer flask.

One millilitre of serum was diluted to 20 ml. with one of the solutions to be described, thoroughly mixed, and passed through No. 1 Whatman filter paper. The fluorescence of a 15 ml. portion of the filtrate was determined by means of a Coleman photofluorometer with standard 'Vitamin B_1' filters transmitting active light of wave-length 365 mμ. The photofluorometer was standardized by adjusting the instrument to give a scale reading of 50 for a solution containing 0.20 γ of quinine sulphate per ml. (5).

Effect of Diluents

During preliminary trials it was observed that when sera from fresh and spoiled samples of salted butter were diluted with 10% potassium chloride solution their fluorescence values differed by 38 photofluorometer units. These sera were turbid, presumably owing to dispersion of fat particles. It was believed that fat solvents mixed with other diluents for the serum might eliminate this difficulty. However, mixtures of dioxane, acetone, and chloroform with various salt solutions proved unsatisfactory. A number of diluents when used by themselves showed promise and merited further investigation.

An aqueous solution of sodium acetate was found to be the most satisfactory solvent because it gave the greatest difference in fluorescence readings between fresh and spoiled butter (viz., 45.0 and 65.6, respectively) and the solution was only slightly turbid. The sodium chloride, ammonium chloride, and ethyl alcohol solutions gave satisfactory differences but were too turbid. Sodium acetate, sodium chloride, ethyl alcohol solutions, and water were selected for further study.

Effect of pH

Hydrogen ion concentration has been shown to have an effect on the fluorescence of an extract of defatted dried egg powder (6). In the present study, pH effects were evaluated using the selected diluents mentioned above. Water and solutions of sodium chloride and ethyl alcohol were adjusted to the desired pH by adding dilute hydrochloric acid or sodium hydroxide solution; the pH of the sodium acetate solution was adjusted with dilute acetic acid or sodium hydroxide.

The diluted sera from spoiled butter increased in turbidity with increase in pH between 4 and 9, while that from fresh butter remained fairly clear. At pH 2 all the diluted sera were quite clear but fluorescence values were small. Sera, diluted with sodium acetate solution adjusted to pH 5, resulted in the clearest extracts, and a large difference in fluorescence values between fresh and spoiled butter (viz., 16.0 and 87.0, respectively). Therefore, a 10% solution of sodium acetate adjusted to pH 5–6 was selected as an appropriate diluent and used in all subsequent work.

Effect of Temperature

It was observed in the above study that duplication of results was poor in some instances. Since it had been demonstrated that temperature affected

the fluorescence of extracts of dried whole egg powder (6), it was considered that temperature variations might be responsible for the difference between duplicates. To evaluate this the procedure was modified as follows: samples of butter placed in centrifuge bottles and heated in a boiling water-bath were centrifuged when the samples were at each of the following temperatures: 35°, 40°, 50°, 60°, and 80° C. Fluorescence values were determined for each sample as previously described.

The results showed that temperature had little effect on the fluorescence of sera from fresh butter, but an increase in temperature caused a small decrease in fluorescence of sera from spoiled butter. The greatest difference in fluorescence values between fresh and spoiled butter (viz., 26 0 and 35 0, respectively) was obtained for sera separated by heating to 40° or 50° C. Hence heating to 45° C. in a boiling water-bath prior to centrifuging was believed desirable.

Effect of Dilution

The fluorescence of a solution may be quenched by various factors, such as too great a concentration of fluorescing substance, which may be avoided by proper dilution. Such procedures may introduce other errors, namely, quenching or apparent quenching due to the solvent or instrument error (10). Therefore, it was of value to determine behaviour of butter sera diluted to various concentrations.

One-millilitre aliquots of serum, obtained from a sample of spoiled butter, were diluted to the following volumes with 10% sodium acetate solution (pH 5-6): 20, 30, 40, 50, 60, 70, 90, 100, and 200 ml. Fluorometric readings on these solutions are shown in Fig. 1. At the higher concentration there is slight quenching, as the relation deviates from the linear. Therefore solutions should be diluted so that readings will fall between 10 and 70 fluorescence units.

Stability of Diluted Sera

The time elapsing between dilution of a serum and photofluorometric measurement might conceivably affect the fluorometric value obtained. To study this, serum obtained from spoiled butter was diluted 1 : 50 with 10% sodium acetate of pH 5-6, allowed to stand at room temperature, and fluorometric values determined at intervals throughout a six-hour period. The data show that the diluted serum is reasonably stable at room temperature, since there is a decrease of only five fluorometer units (viz., 45.0 to 40.0) in six hours.

Recommended Procedure

As a result of the above observations the following procedure was adopted as being most suitable. Whole salted butter (125 gm.) weighed into a centrifuge bottle was heated to 45° C. with constant stirring in a boiling water-bath,

and centrifuged for 10 min. at 1700 r.p.m.   Fat and serum were separated by
centrifuging and siphoning off the fat.   Two millilitres of clear serum was
diluted to 50 ml. with 10% sodium acetate solution (pH 5–6), mixed, and
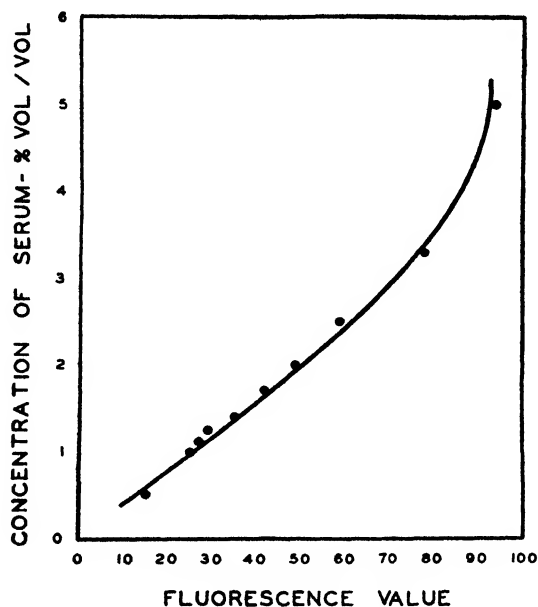filtered through No. 1 Whatman filter paper.   The fluorometric value of the

FIG. 1.   *Effect of dilution on the fluorescence value of spoiled butter serum.*
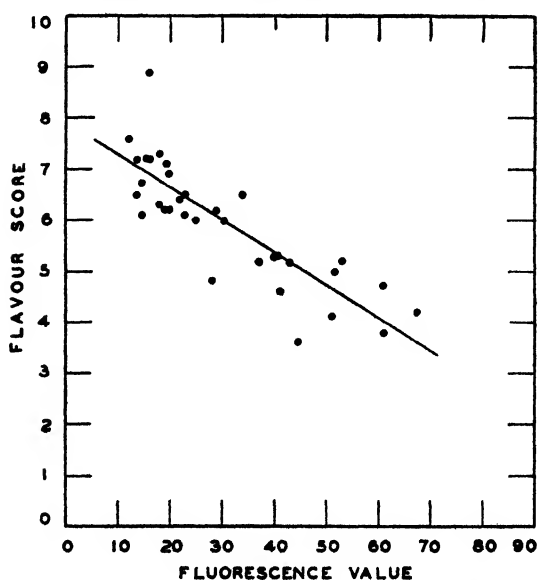
FIG. 2.   *Relation between flavour score and fluorescence values on samples of stored butter.*

filtrate was determined in a Coleman photofluorometer. As the diluent had a small fluorescence value it was necessary to correct all fluorescence readings.

Evaluation of the Method on Stored Butter

Materials and Methods

The material investigated consisted of four sets of samples of canned and printed salted butter from an eastern and a western Canadian creamery. The butter was stored at 32.2° C. (90° F.) for 32 days and sampled at intervals to give a wide range of quality. To assess the suitability of the fluorometric method, fluorescence values were compared with flavour scores. The usual method for scoring butter was not employed as it is not readily adaptable to statistical treatment. Butter was scored as follows: 10, excellent; 8, good; 6, fair; 4, poor; 2, bad; 0, inedible. The ten tasters were required to score a set of four samples chilled to approximately 10° C. (50° F.).

Results

Fluorescence values increased with a decrease in flavour score (Fig. 2). Good agreement is indicated between the two ($r = -.84$). The equation for these data is:

$$y = 7.867 - 0.0632\, x,$$

where x = corrected photofluorometer readings and y = flavour score. It is evident that fluorescence values of 30 and 61 correspond to flavour scores of 6 and 4, respectively.

Statistical analyses of the data obtained for each sample of butter showed a high correlation and no difference between regression coefficients. There was no significant increase in correlation by using the log of fluorescence values, which is the usual form of curve to be expected. However, this may possibly be due to insufficient samples of low and high flavour scores. The co-linearity of the four sets of data was slightly different. While this difference was statistically significant it is probable that the taster level of scoring did not remain constant.

Discussion

The high correlation and lack of significant difference in regression coefficients between fluorescence measurements and flavour scores indicate that this test should be a valuable aid in assessing the keeping quality of butter under unfavourable storage temperatures. However, this test will not assess flavour deterioration in butter due to tainting by foreign materials, nor can it be definitely stated whether it will apply to other forms of spoilage that may occur in commercial practice (1, pp. 75-92).

Acknowledgments

References

1. HAMMER, B. W. and COLLINS, M. A. Iowa Agr. Expt. Sta. Research, Bull. No. 169. 1934.
2. KRIENKE, W. A. and FAUTS, E. L. Assoc. Southern Agr. Workers, Proc. Ann. Convention, 43 : 121. 1942.
3. MARSHALL, J. B., GRANT, G. A., and WHITE, W. H. Can. J. Research, F, 23 : 286-294. 1945.
4. PEARCE, J. A. Can. J. Research, C, 21 : 57-65. 1943.
5. PEARCE, J. A. and THISTLE, M. W. Can J. Research, D, 20 : 276-282. 1942.
6. PEARCE, J. A., THISTLE, M. W., and REID, M. Can. J. Research, D, 21 : 341-347. 1943.
7. RITTER, W. and NUSSBAUMER, T. Schweiz. Milchztg. 64 : 465-466. 1938.
8. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 211-222. 1943.
9. WILEY, W. J. J. Dairy Research, 10 : 300-309. 1939.
10. WOKES, F., ORGAN, J. G., STILL, B. M., and JACOBY, F. C. Analyst, 69 : 1-5. 1944.